




REVIEW PAPER

Kinga Szlachetka ^{1(ABDFG)}, Paulina Kut ^{1(ABDFG)}, Agnieszka Stępień  ^{1,2,3(ABDFG)}

Cytotoxic and anticancer activity of *Moringa oleifera*

¹ Dietitians' Scientific Club section Food safety at Department of Dietetics, Institute of Health Sciences, College of Medical Sciences, University of Rzeszow, Rzeszów, Poland

² Centre for Innovative Research in Medical and Natural Sciences, Laboratory of Innovative Research in Dietetics, College of Medical Sciences, University of Rzeszow, Rzeszów, Poland

³ Department of Dietetics, Institute of Health Sciences, College of Medical Sciences, University of Rzeszow, Rzeszów, Poland

ABSTRACT

Introduction. Given its very strong antioxidant properties, *Moringa oleifera* is particularly noteworthy among medicinal plants. The high contents antioxidants in the *M. oleifera* determining her antioxidant activities deciding for very important anticancer properties.

Aim. The aim of the paper is to provide an overview of the cytotoxic and anticancer activity of *Moringa oleifera*.

Material and methods. This review was performed based systematic analysis of literature.

Analysis of the literature. The results of scientific research conducted *in vitro* indicate that extracts from *Moringa oleifera* significantly affect the development of human cancer cells such as myeloma, leukemia, cervix, breast, colon, lung, liver, neuroblastoma, pancreas, colorectal, epidermoid, oral, ovarian, muscular, prostate, skin.

Conclusion. This indicates *Moringa oleifera* as that they may be used as a therapeutic agent to support oncological therapies.

Keywords. anticancer activity, cytotoxic activity, *Moringa oleifera*

Introduction

Owing to their highly-valued curative properties, herbaceous plants have been used in folk medicine. Medicinal effect of herbs depends on their contents of bioactive compounds with antioxidant properties.

Antioxidants, substances known for their health promoting properties, neutralise excess free radicals, this way preventing oxidative stress in the organism. Long-lasting oxidative stress increases a risk of inflam-

mation which causes numerous diseases, e.g. cancer, heart failure, circulatory system disorders, and many more, that is why antioxidants are so important in preventing these conditions. Medicinal plants are a natural source of antioxidants, such as polyphenols, flavonoids, terpenes, saponins and azulenes.¹

Given its very strong antioxidant properties, *Moringa oleifera* is particularly noteworthy among medicinal plants.

Corresponding author: Agnieszka Stępień, e-mail: astepienurz@gmail.com

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Moringa oleifera (synonyms: *Guilandina moringa* L., *Moringa moringa* (L.) small, *Moringa pterygosperma* Gaertn., MO, *Moringa oleifera* Lam), also referred to as horseradish tree or miracle tree, drumstick tree, ben oil tree, benzolive tree, moringa is the most commonly cultivated tree representing the monogenetic family *Moringaceae*; it reaches approximately 10 metres in height and is grown in Africa, Asia and Latin America (fig.1.). All parts of the tree are edible and they have high contents of amino acids, vitamins, and minerals, such as calcium, potassium, zinc, magnesium, iron, and copper.²⁻⁵ Particularly notable, however, are the bioactive compounds contained including vitamins A, C and E, which are strong antioxidants, as well as polyphenolic compounds – quercetin and kaempferol, terpenoids, glycosides, tannins and saponins.⁶⁻¹⁴ The high contents this antioxidants in the *M. oleifera* determining her antioxidant activities deciding for antiplatelet, hypotensive, antimicrobial, antiulcerous, anti-inflammatory, hypocholesterolemic, and hypoglycemic properties, and very important anticancer properties.¹⁵⁻²³



Fig. 1. Dried herb leaves *Moringa oleifera* (photograph by Kinga Szlachetka)

Numerous scientific studies have shown that *M. oleifera* contains many bioactive compounds, among others alkaloids, flavonoids, and phenolic compounds determining the antioxidant and anti-cancer. These bioactive ingredients cause changes in the cycle of cancer cells. By inducing in the cell the mechanism whose main element is the pathway covering the process of apoptosis, i.e. programmed cell death causing cell contraction, DNA fragmentation, cell membrane changes, chromatin, consequently leading to death of the apoptotic cell. Antioxidants also have an effect on inhibiting the proliferation of cancer cells. Molecular change observed in cancer cells is a decrease of the proliferation (cytostatic effect) and the like and decrease in cell survival rate due to the induction of apoptosis (cytotoxic effect) significantly affecting the properties of anti-cancer and cytotoxic *M. oleifera*.^{18,24,25}

Aim

In this paper, we present the results of an analysis of the scientific literature of *in vitro* studies confirming the cytotoxic and anti-tumor properties of *Moringa oleifera* resulting from the ability to inhibit proliferation, induce apoptosis and cell cycle arrest.

Analysis of the literature

Parvathy and Umamaheshwari investigated the effects of extract from *Moringa oleifera* leaves in human multiple myeloma cell lines (U266 B1 human B-lymphocyte plasmacytoma). The cells were incubated with MO leaf extract dissolved in methanol, ethanol, ethyl acetate and chloroform, and cytotoxicity testing was performed using neutral red dye uptake assay. The findings showed that methanol extract was characterised by the highest cytotoxic activity related to these cells. It was observed that even its small quantity significantly inhibited proliferation of these cells, which suggests its high anti-cancer activity.²⁶

Another study also reports anticancer effect of *Moringa oleifera* leaf extract observed in primary cell lines of acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL), collected from patients, as well as a line of hepatocarcinoma cells (HepG2). Khalafalla et al. in their study identified anticancer effects of hot water, cold water and 80% ethanol extracts of moringa leaves in the primary cells of the two types of leukaemia and liver cancer. The findings related to cytotoxicity of these extracts, determined using MTT (the tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, showed the highest activity of the ethanolic extract with respect to both AML and ALL cells, while the hot water extract was more active than the cold water extract. In the case of HepG2 cells, hot water extract showed the strongest anticancer activity.²⁷

A study by Varalakshmi and Nair examined anticancer properties of aqueous, methanol and hexane extracts of *Moringa oleifera* leaves with respect to cervix cancer cell line (HeLa) and normal human lymphocytes. Following incubation with *M.oleifera* extract, antiproliferative effects were assessed using MTT assays and trypan blue dye. Based on DNA fragmentation analysis performed using acridine orange–ethidium bromide (AO/EB), they identified apoptotic effect of these extracts. The findings showed good cytotoxicity of aqueous extract, depending on its concentration, with respect to cancer cells, compared to methanol and hexane extracts. However, aqueous extract produced the lowest cytotoxic effects in lymphocytes.²⁸

Sreelatha et al. also evaluated the effects of an aqueous extract from *M. oleifera* (MO) leaves on a human tumor (KB) cell line derived from glandular cancer of cervix. After incubation of the KB cell culture with MO extract (0-200 ug/ml), based on the cell viability assess-

ment (MTT test) they showed its inhibitory effect on the cell proliferation increasing with concentration. The anti-proliferative effect of this extract resulted from the induction of apoptosis and morphological changes of cells determined by DAPI and propidium iodide staining. The authors point to the strong antiproliferative effect of *M. oleifera* leaf extract on cells of this type of cancer.²⁹

In another study described the effects of *Moringa oleifera* seed methanolic extract on human cancer cell lines such as lung (A-549), liver (Hep-2), colon (502713 and HT-29) and neuroblastoma (IMR-32). The cytotoxicity of this extract (100 µg/ml) was determined by these sulforhodamine B (SRB) dye test on this tumor cell lines. Growth inhibition was observed in the lung, colon HT-29 cell line and neuroblastoma 80%, 95% and 93% respectively. In contrast, the results showed no cytotoxicity to the Hep-2 line, and the maximum for the colon cell line 502713.³⁰

Likewise, a study by Tiloke et al. provided supporting evidence showing anticancer effects of aqueous extract of *Moringa oleifera* leaves in human cell line of lung cancer (A549). Following 24 h incubation of human cancer cell lines with MO extract the researchers assessed the level of oxidative stress (TBARS method) and the level of glutathione. Proapoptotic effect of aqueous extract of MO, on the other hand, was reflected by significantly increased expression of p53 protein and mRNA p53.³¹

In addition, subsequent studies determined the antitumor properties of the *Moringa oleifera* aqueous leaf extract against two human pancreatic cancer cell lines (Panc-1 and COLO-357). It was determined by means of colorimetric analysis and flow cytometry that MO extract inhibited the growth of pancreatic cancer cells of both tested lines. Test results showed that the highest inhibition of Panc-1 and COLO-357 cell growth was observed at a concentration of ≥ 0.75 mg/ml extract.³²

Charoensin et al. investigated anticancer properties of *M. oleifera* leaves in relation to human cell lines of hepatocarcinoma (HepG2), colorectal adenocarcinoma (Caco-2) and breast adenocarcinoma (MCF-7). Cytotoxicity of methanol and dichloromethane extract with respect to cancer cell lines was assessed using MTT assay, and chemoprevention was examined using quinone reductase induction testing. The findings showed greater anticancer effect of dichloromethane extract in the cells of Hep G2 and MCF-2 as well as greater chemopreventive effect.³³

In subsequent studies, Jung et al. have shown anti-tumor activity of *Moringa oleifera* leaf extract against human cancer line lung (A549). They observed that an aqueous extract (300 mg/ml) of MO strongly induced apoptosis, inhibited the growth of cancer cells and lowered the level of internal reactive oxygen species (ROS) in human lung cancer cells.³⁴

Activity of *Moringa oleifera* leaves and bark in relation to human cancer cell lines of breast (MDA-MB-231) and colorectal (HCT-8) was investigated by Al-Asmari et al. They determined that the extracts, by inducing apoptosis and inhibiting cell cycles, produced phenotype changes in the cells of both cancers and cell death.³⁵

Similarly, a study carried out by Jung presented evidence for antiproliferation effect of extract from *Moringa oleifera* leaves. Following incubation of human cell line of hepatocellular carcinoma (HepG2) with aqueous extract of *Moringa* leaves, flow cytometry was applied to assess effects in DNA content and cell cycle stages. It was found that the extract induced apoptosis of cancer cells.³⁶

Other studies have determined the antitumor effect of extracts (n-hexane, chloroform, ethyl acetate, 50% methanol) from *M. oleifera* leaves and 15 fractions of ethyl acetate extract (F1 to F15) against human epidermoid cancer cell line (Hep2). After incubation, cell viability was assessed by sulforhodamine B staining. Among all tested MO extracts and fractions, the isolated F1 fraction showed the highest cytotoxic activity against tumor cells. It was found through the use of *High-Performance Thin-Layer Chromatography* (HPTLC) technique that F1 contains a large amount of antioxidants – phenolic compounds that affect these properties.³⁷

Kaur et al. analyzed the chemical composition of the obtained methanolic extract from *M.oleifera* leaves. They isolated and identified on the basis of melting point, Nuclear Magnetic Resonance (13C-NMR, 1H-NMR), Infrared Spectroscopy (IR), Fast Atom Bombardment Mass Spectrometry (FAB-MS) chemical compound β -D-glucopyranosidetetradecanoate belonging to the group of antioxidant flavanoids. They then evaluated its cytotoxic activity against human cancer cell line colon (Colo-320DM), oral (KB-403), ovary (PA-1), breast (MCF-7). After incubation, the cytotoxicity of this compound was assessed by the MTT test on the tested tumor lines. Inhibition of cell growth of the Colo-320 DM line was observed already at a concentration of 2.52 µg/ml of this flavonoid, while for KB-403 at 3.62 µg/ml, for Pa-1 at 6.46 µg/ml and MCF-7 at 10.00 µg/ml. The results indicate that this compound has the highest cytotoxicity against colon cancer compared to the other 3 lines.³⁸

Milungo et al. examined the antiproliferative activity of extracts (50% methanol in dichloromethane) from *Moringa oleifera* leaves against human liver (Hep-G2) and muscular (RD). After incubation with the MO leaf extract, tumor cell viability (crystal violet staining and Optical Density measurement) was assessed. Significant inhibition of cell growth of both tumor lines was observed already at 0.017 mg/ml extract concentration for the RD line and at 0.50 mg/ml higher concentration for Hep-G2.³⁹

In subsequent studies, researchers analyzed the effect of *M.oleifera* leaf extract on human cancer cell lines ovarian (A2780CP20) and prostate (PC3). By using colorimetric analysis with the AlamarBlue dye after exposure of both tumor cell lines with extract, their viability was assessed. It was observed that the concentration of the extract inhibiting the growth of A2780CP20 cells was 0.27 mg/ml and for prostate cancer cells 0.17 mg/ml.⁴⁰

Madi et al. presented the results of research on the antitumor activity of *Moringa oleifera* leaf extract against human cancer cell line lung (A549), liver (Hep-G2), colon (CaCo2), leukemia-associated T cells (Jurkat). All tumor cell lines were incubated with an aqueous extract (dried leaf powder soaked in hot water) of varying concentration (0.05-2.5%). Subsequently, after incubation, cell viability was determined by MTT test and the anti-proliferative effect of MO extract on them was assessed. It was observed that the MO extract caused a decrease in the viability of all tested cell line types depending on the concentration of the extract. However, the most cytotoxic effect was already determined at 0.05% of the extract used versus A549 compared to Jurkat, Caco2, Hep2 at 0.1-0.4%.⁴¹

Guon and Chung conducted scientific studies to evaluate the effect of *Moringa oleifera* fruit extract on human melanoma cells (A2058). It was observed thanks to the MTT test that the *Moringa oleifera* fruit extract significantly inhibited the viability of A2058 cells and propagated their apoptosis depending on the concentration (0-200 µg / ml). It was determined that at concentrations of 150 and 200 µg/ml MO extract there was a significant reduction in the proliferation of these cells, to 11.3 and 10.1%, respectively. Thanks to the double staining test for Annexin V-PI to distinguish the stages of apoptosis, it was found that *Moringa oleifera* fruit extract effectively induced mitochondrial apoptosis of A2058 cells.⁴²

Subsequent results of the conducted tests showed the cytotoxic properties of *Moringa oleifera* Lam leaves against the colon cancer cell line (HCT116). The obtained methanolic MO leaf extract was fractionated by column fraction chromatography (MOL1-MOL4). It was determined by MTT and Western blotting that the fractions showed high antiproliferative activity against HCT116 cells especially MOL2, MOL3 and MOL4.⁴³

Adebayo et al. analyzed the cytotoxic activity of *Moringa oleifera* seeds on a breast cancer cell line (MCF7). The obtained aqueous and ethanol extracts from MO seeds were divided into 4 fractions (hexane solvent, dichloromethane, chloroform and n-butanol). It was determined by MTT that all fractions showed high antiproliferative activity on MCF7 cells. The MO seed extracts analyzed inhibited the multiplication of MCF7 cells, aqueous at 280 µg/ml, respectively, and the hexane fractions 130 µg / ml and dichloromethane, respectively 26 µg/ml.⁴⁴

Jinghua et al. investigated antioxidative potential of extract from MO leaves and they described molecular mechanisms associated with its anticancer activity. Following incubation of human cell line of colon cancer with hexane extract from MO leaves, the researchers assessed toxicity using MTT assay, and examined apoptotic effects by measuring caspase activity. The findings showed cytotoxic effects of extract from MO leaves in these cancer cell lines. The study demonstrated that extract from leaves of *Moringa* significantly inhibits proliferation of colon cancer cells and induces cell death via mitochondrial pathway of apoptosis. The research shows the potential for using extracts from MO leaves in prevention and treatment of this type of cancer.⁴⁵

In their research, Ndungu et al. evaluated the cytotoxic activity of *Moringa oleifera* leaf extracts against human breast cancer cell lines (HCC 1395), prostate (DU145) and cervix (Hela) and the non-cancer cell line (Vero). The presence of alkaloids, terpenoids, tannins, flavonoids, glycosides, phenols and saponins was determined in MO extracts. The effect of MO on the inhibition of tumor cell growth was assessed by MTT. It was observed that the methanol-dichloromethane extract of *M. oleifera* was characterized by higher activity than aqueous. All MO extracts showed antiproliferative activity against all cancer cell lines tested, and for the DU145 line already at 66.290 µg / ml. In contrast, *M. oleifera* extracts were not cytotoxic to Vero cells.⁴⁶

However, Ali and et al. in their study they evaluated the cytotoxic activity against the human cancer cell line breast (MCF-7) extract from *Moringa oleifera* leaves. The study reported exposure of MCF-7 cell lines with seven types of extracts obtained. MO leaf extracts were obtained using two methods: extraction (ethanol, 2-propanol, acetone, petroleum ether, water-solvent) and soaking (ethanol, boiling water-solvent). Only the obtained extract (water as solvent extraction techniques) 81.77 ± 6.05 µg/ml showed cytotoxic activity against MCF-7 among all extracts used.⁴⁷

In their research, Vipul and Vishal analyzed the anti-tumor effect of the extract (water: methanol, 30:70 v / v) of *Moringa oleifera* leaves on human cancer cell lines, prostate (DU-145), breast (MCF-7), hepatoma (HEP- 3B), myelogenous leukemia (K-562) and colorectal (HCT-15). It was determined by MTT that MO leaf extract induced dose-dependent inhibition of K-562 (32.43 µg/ml), DU-145 (42.74 µg/ml), and HCT-15 (5.21 µg/ml) cell proliferation), MCF-7 (24.76 µg / ml), HEP-3B (29.37 µg / ml). It was also determined that at these concentrations a significant fragmentation of DNA fragmentation for K-562, DU-145 and HCT-15 cell lines.⁴⁸

Whereas Ju et al. focused their research on assessing the properties of the extract (water: ethanol, 20:80 v/v) of *Moringa oleifera* flowers against human prostate cancer (PC-3) cell lines. Observed thanks to the MTT test

and assessment of cell cycle progress using flow cytometry that this MO extract had significant activity on PC 3 cells at a dose of 6.25 µg / ml (48 h). It induced G1 phase cycle arrest and apoptosis as confirmed by Annexin V staining, and detected markers of immunoblot apoptosis lead to increased protein expression in caspase-3 activity, indicates induction of apoptosis. Researchers based on the obtained research results that extracts of *Moringa oleifera* flowers lead to phosphorylation / activation of AKT, which affects the upregulation of the BCL-2 and BCL-XL prosurvival genes; which binds and inhibits caspase 3/7/9 required for induction of apoptosis in prostate cancer.⁴⁹

Research team Podesta et al. evaluated the effects of *M. oleifera* aqueous leaf and seed extract on human cell line lymphoid (Jurkat E6-1), monocytic leukemia patient (THP-1) and peripheral blood mononuclear cells (PBMC) from healthy donors. The results of this study showed antiproliferative assessment by the trypan blue and proapoptotic effect of aqueous extracts obtained from *M. oleifera* leaves and seeds on tumor lines but not against peripheral blood mononuclear cells (PBMCs). The proapoptotic effect of the MO seed extract (MOE-S) was associated with decreased expression of B-lymphoma protein (BCL2) and sirtuin-1 (SIRT1), which are involved in apoptosis.⁵⁰

In other studies, the cytotoxic activity of the obtained ethanol extract from *M. oleifera* leaves and then fractionated with n-hexane against human breast cancer cell line (T47D) was determined. Hexane fraction MO extract (hMO) was shown at 235.58 µg/ml cytotoxic effect and inhibition of T47D cell proliferation. It was observed that the apoptosis of these cells determined by flow cytometry induced their slow death and cell cycle arrest at the G0-G1 and G2-M phases. However, thanks to immunocytochemical tests, hMO has been shown to reduce the expression of anti-apoptosis protein, Bcl-2 and cell cycle regulating protein, cyclin D1, in a concentration-dependent manner.⁵¹

Further researchers Tiloke et al. focused on assessing the apoptosis-inducing effect of an aqueous MO leaf extract on human liver hepatocellular carcinoma (HepG2) cells. Exposure of HepG2 cells to an aqueous MO extract resulted in a decrease in viability at 4.48 mg/ml (MTT test). It was also observed that it significantly increased lipid peroxidation (TBARS test), DNA damage (comet test). It was determined that in their cell cycle a significant decrease in G1, S and G2-M phases due to flow cytometry. Increased caspase-9, -3/7 activity was determined by luminometry, with a significant decrease in ATP levels indicative of apoptosis. And thanks to western blot analysis, a significant reduction in c-myc, p-Bcl2 and Hsp70 protein expression and a significant increase in Bax, Smac/DIABLO and PARP-1 cleavage also confirmed the pathway of apoptosis. Numerous re-

sults of the above studies indicate that MO water extract has the ability to induce cell cycle arrest and apoptosis in HepG2 cells.⁵²

Do et al. evaluated the effect of the aqueous extract of *Moringa oleifera* Lam leaves. human malignant melanoma cells (A375), human metastatic melanoma cell (A2058) focusing on the assessment of molecular mechanisms (preventing proliferation, induction of apoptosis). It was determined by the WST-1 test that MO extract inhibited the growth of A375 cells more than A2058 cells in a dose-dependent manner (0 to 200 µg/ml). This cytotoxicity of the MO extract towards the A375 line decided that the researchers conducted further analyzes only for it. It was confirmed by Annexin V FITC test that MO extract induces apoptosis of A375 cells. The study results also showed that the MO extract increased the Bax / Bcl-2 ratio, decreased the mitochondrial membrane potential, and activated caspase 3/7, caspase 9, PARP and AIF translocation which led to apoptotic cell death.⁵³

Conclusion

The results of scientific research presented in this review suggest the *Moringa oleifera* may be highly valued in medicine for its her the properties anticancer and cytotoxic. This suggests that *M.oleifera* could be used in the prevention and treatment of various types of cancer. However, it is necessary to conduct additional clinical tests involving human subjects, in order to confirm the positive contribution of *M. oleifera* in treatment of cancer, and it is equally important to assess its interactions with medication administered to patients with these conditions.

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