REVIEW ARTICLE

Moringa oleifera: Antioxidant, Anticancer, Anti-inflammatory, and Related Properties of Extracts in Cell Lines: A Review of Medicinal Effects, Phytochemistry, and Applications

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Abstract

Moringa oleifera (MO), or the common drumstick possesses antioxidant properties, and its pods, seeds, leaves, and bark have been used for the treatment of inflammatory and cancerous conditions. This systematic review attempts to synthesize original studies of MO extracts in cell lines to determine their specific antiproliferative, antioxidant, anti-inflammatory, and related effects. The literature was obtained from PubMed central, the Cochrane registry, and other sources including Google Scholar, and Embase. Studies fulfilling the inclusion criteria were selected. Custom data collection forms were employed and two independent evaluators compiled the relevant information. Eighteen studies were selected after applying inclusion and exclusion criteria. In most studies, MO leaves had more potent properties compared to other parts of the plant. Ethyl acetate and ethanolic extractions improved the potency of the extract. Effects were selective (different for normal cells and cancer cells) and dose-dependent. Anticancer and antioxidant activities were consistently reported, with effects exerted at the genetic and molecular levels. MO extracts potentially could be employed for therapeutic applications. The optimal sources, preparation protocols, and dosages have been researched, though further scrutiny is needed for a comprehensive formulation.

Keywords: Anticancer, Anti-inflammatory, Antioxidant, *Moringa oleifera*.

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Introduction

Herbal preparations have been used by humans since time immemorial for treating various medical disorders. More than half a million unique species of plants on the earth present a rich resource of phytochemicals with potential therapeutic properties. Secondary metabolites are produced by plants as a defense against injurious pathogenic and environmental conditions. The major advantage of synthetic chemicals is that they are relatively much safer, easily accessible, and cost less. Initially exploited for antimicrobial use, the applications have expanded to a wide range of conditions including cancer, inflammatory disorders, immune disorders, and diabetes.[1–3]

Moringa oleifera (MO) is a common plant species of mainly tropical climes. Almost every part of the plant has been employed for nutritional and medicinal uses. Traditional medicine and now increasingly, modern science have recognized the unique composition of this plant and its efficacy in the treatment of various diseases. In particular, the extracts of the plant are known to possess antiproliferative, anti-inflammatory, antimicrobial, antioxidant, and osteoprotective effects.[4–13]

Oxidative stress is a pathological consequence of excess free radical activity in the body. Normally there is a balance between the beneficial and the toxic properties of free radicals, especially reactive oxygen species (ROS). There is increasing evidence that this balance is disturbed by conditions that unfortunately are part of modern life. Processed food, unhealthy lifestyle, including sedentary state (or excess exercise), deleterious habits, mental stress, and pollution, have played havoc with our physiology. Some of the major conditions the ROS plays a role in include cancer, inflammatory conditions like arthritis, cardiovascular and neural disease, aging, autoimmune diseases, and many other disorders. To counteract free radical damage, the body systems generate free radical scavengers or antioxidants that neutralize their potency. Exogenous antioxidants abound in nature, mainly present in plant-based sources like fruit and vegetables.[14–16]

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In the last three decades, there has been considerable interest in the applications of natural products in the treatment of diseases. There is recognition of the fact that plant medicine as practiced traditionally in many cultures is extremely effective in treating many conditions that have been eluding allopathic systems. There have been numerous studies of plant extracts to determine their beneficial effects in the treatment of diseases.[17–34] The present systematic review attempts to synthesize the results and conclusions of validated studies on the antioxidant, anticancer, anti-inflammatory, and related effects of *M. oleifera* extracts in cell lines. This would consolidate the current evidence of the effects of MO extracts in live cell cultures. There is a specific focus on objective conclusions derived from laboratory techniques like cell proliferation assay, cytotoxicity assay, oxidation stress assay, PCR, and gene analysis of inflammatory and cancer genes.

Materials and Methods

Sourcing and Study Selection

Online evaluation of electronic literature (PubMed/Medline database and the Cochrane Study register, including Embase) was performed. Keywords applied were "*Moringa*" and "*Moringa oleifera*." Original articles in journals with good credentials were preferred. To further select validated *ex vivo* research, studies that employed cell lines were preferred. English language articles were collected from relevant scientific journals dated 2000–2020 (20 years). Table 1 summarizes the selection criteria and Flowchart 1 summarizes the PRISMA protocol employed.

Data Collection

The data extraction form customized for this review is presented in Table 2. All relevant information for the analysis was included. Two independent observers were employed. The final report was generated by consensus.

Data Items

Data items included variables were setting/country, MO extract types, methodology (the type of cell lines, laboratory procedure), analysis, results, and conclusions (Table 2).

Bias Assessment

The risk of bias in selected studies was assessed employing the Cochrane tool (Cochrane Handbook v 5.1.0, 2011).[35]

Qualitative Synthesis

This was performed following established guidelines (Thomas and Harden and Bearman and Dawson).[36,37] Parameters were plant source (leaf, bark, seed), type of extract (aqueous, ethanolic, fractionated), cell line, effect studied, and other relevant factors including dosage.

Table 1: Inclusion and exclusion criteria

Inclusion criteria	*Exclusion criteria*
Clinical trials registered in Cochrane Library, PubMed listed articles mentioning the use of *Moringa*/*Moringa oleifera* in anti-inflammatory, anticancer, antioxidant, and related effects	Trials deemed to be unsuitable for our analysis due to lack of adequate information and major deficits in study design, methods, etc. Studies related to diabetes mellitus and antibacterial effects
Studies only on cell lines of human or animal sources, both normal and tumor types	Studies that include *Moringa*/*Moringa oleifera* in other applications like non-medical uses, veterinary uses, Animal studies
Publications ranging from the year 2000 to 2020	Studies earlier than 2000
English language articles (including translations)	Other language articles

Flowchart 1: Process of study selection as per PRISMA guidelines

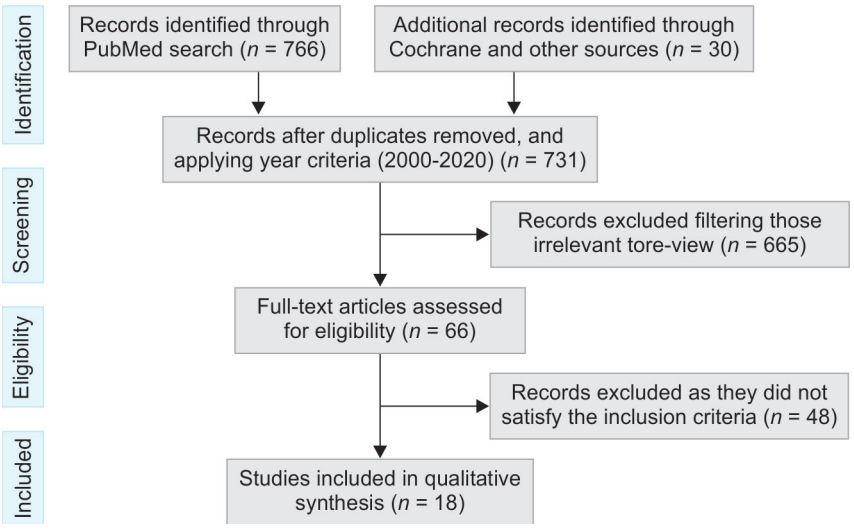

Table 2: Collected data from selected clinical trials

Trial	*Setting/ country*	*Type of cell line*	*Major effect studied*	*Type of MO extract*	*Laboratory procedure*	*Results*	*Conclusion*
Tiloke et al. (2013)	South Africa	A549 cancerous lung alveolar epithelial cells	Antiproliferative	Aqueous leaf extract (hot-treated)	Oxidation stress assay (TBARS, glutathione) Comet assay Caspase assay Western Blot, qPCR (mRNA exp of p53 and Nrf2)	Increased p53 and caspase activities, with increased oxidative stress (due to down regulation of Nrf2).	Oxidative stress-induced apoptosis in cell line implying potent antiproliferative activity.
Jung (2014)	Korea	A549, H23, and H358 MCF-7, A431, HT1080, COS-7 African green monkey kidney cell line)	Antiproliferative	Aqueous leaf extract (cold treated)	MTT proliferation assay, flow cytometry, colony assay, PCR, Western Blot, microarray	Upregulation of caspase 3 and increased apoptosis of A549. Inhibition of ROS. Downregulation of oncogenes and induction genes due to rRNA degradation. The effect was significantly lower in normal cell line.	Antiproliferative activity in cancer cell lines, (cold-treated extract may be superior to heat-treated). Possible sparing of normal cells.
Al-Asmari et al. (2015)	United States	MDA-MB-231 (breast) and HCT-8 (colorectal) cancer cell lines	Antiproliferative	Ethanolic extract of leaf, bark, and seeds (Soxhlet technique) Heat treatment could be a limiting factor	Motility assay Clonogenic survival assay Cell viability, apoptosis, and cell-cycle assay	Decreased cell survival with leaf and bark, but not as much with seed extract. Reduced motility, colony formation in leaf and bark extracts. Late apoptosis and G2-M phase arrest were observed in leaf and bark extracts.	Antiproliferative activity in leaf and bark extracts. Seed extracts not effective. Many anticancer compounds are identified in extracts.
Adebayo et al. (2017)	Malaysia	MCF7 breast cancer cell line, MCF 10A (control)	Antiproliferative	Crude and fractionated ethanolic extracts of seeds	Cell viability assay using aqueous extract (CWE), alcoholic extract (CEE), hexane fraction (HF), dichloromethane fraction (DF), etc	Cell viability significantly reduced in CWE, HF, and notably DF extracts. In a normal cell line, only HF showed the least cytotoxicity. Other extracts showed high cytotoxicity of both cancer and normal cells.	Hexane fractionated ethanolic extract of MO seed was cytotoxic to the cancer cell and least cytotoxic to normal cells.
Romeo et al. (2018)	Italy	Human PDL stem cells	Neuro-differentiation Adipogenesis, Osteogenesis	Moringin – [4-(*α*-L-rh amnosyloxy) -benzyl isothiocyanate], extracted from *Moringa oleifera* seeds	MTT assay Transcriptome analysis Immunofluorescence of neuronal markers	Improved stem cell proliferation. Early upregulation of neural development genes. No significant effect on oncogenes. Positive IF of markers.	Moringin stimulated neurogenesis in PDL stem cells (important in stem cell therapy) and was not tumorigenic.

(*Contd...*)

Table 2: (*Contd...*)

Trial	*Setting/ country*	*Type of cell line*	*Major effect studied*	*Type of MO extract*	*Laboratory procedure*	*Results*	*Conclusion*
Araujo et al. (2013)	Brazil	NCI-H292 (human pulmonary mucoepidermoid carcinoma) HT-29 (human colon adenocarcinoma) and HEp-2 (human larynx epidermoid carcinoma)	Cytotoxic, anti-inflammatory	Aqueous seed extract, Diluted seed extract, cMoL, WSMoL	Cytotoxicity assay	Aqueous seed extract, cMoL were potentially cytotoxic, diluted seed extract, WSMoL were not. TNFα and IL-1β were reduced. Weak cytotoxic activity esp for HT-29.	Anti-inflammatory effects of these extracts in normal cells. Anticancer effects were not remarkable in the cell lines.
Tan et al. (2015)	Japan	Murine macrophage cell line, RAW 264.7	Antioxidant and anti-inflammatory	80% hydroethanolic extract of *M. oleifera* flower	Cytotoxicity (MTT) assay Assessment of NO, PGE_2, IL-6, IL-1β, TNF-α, NF-κB, iNOS, and COX-2	Minimal cytotoxicity in macrophages, but dose-dependent. Nitrite formation inhibited, PGE_2, IL-1β reduced but IL-10 increased.	MO inhibited NO, pro-inflammatory mediators, but increased anti-inflammatory IL-10 and IκB-α. Evidence of antioxidant and anti-inflammatory activities.
Gothai et al. (2016)	Malaysia	Normal human dermal fibroblasts	Wound healing	Ethyl acetate fraction of MO leaves	MTT assay, proliferation assay, wound scratch assay	No cytotoxicity. Cell proliferation and migration increased in low concentrations but decreased in higher concentrations (>125 µg/mL)	Low doses promote wound healing, the proliferation of normal cells.
Arulselvan et al. (2016)	Malaysia	RAW264.7 (LPS stimulated macrophages)	Anti-inflammatory	*M. oleifera* leaf fractions (butanol, ethyl acetate, chloroform, and hexane)	MTT assay, NO production, ELISA for pro-inflammatory mediators, iNOS, COX-2 IκBα, NFκB	Non-toxic at low concentrations. Inhibition of NO, IL-6, TNFα, IL-1β (esp ethyl acetate fraction). Downreg of iNOS, COX-2. IκBα degradation inhibited, NFκB expression attenuated.	The potent anti-inflammatory effect, esp ethyl acetate fraction. Probably acting on the NFκB pathway.
Jung (2016)	Korea	Human non-small cell lung cancer A549 and human hepatocellular carcinoma HepG2	Antiproliferative	Lyophilized cold MO leaf extract	MTT, colony-forming assay, Annexin V binding, TUNEL assay, Western Blot, Hollow fiber assay (rats)	Dose-dependent cytotoxicity and G1-arrest (apoptosis), colony reduction, Increased proapoptotic and reduced antiapoptotic gene expression, TUNEL positive cells, More cytotoxic to HepG2.	Effective antiproliferative activity, Results of *in vitro* test and oral administration in rats similar, indicating high bioavailability.
Kooltheat (2014)	Thailand	Human monocyte-derived macrophages (MDM) exposed to cigarette smoke extract	Anti-inflammatory	Ethyl acetate fraction of MO leaf and other fractions	ABTS radical cation decolorization Assay, ELISA for TNF, IL-6, and IL-8 Gene analysis using qRT-PCR	Antioxidant activity that did not correlate with phenolic content. TNF, IL-6, and IL-8 were inhibited, as shown by ELISA and gene analysis. Reduced RelA (a subunit of NFκB pathway).	Potent antioxidant and anti-inflammatory activities. Implications in treatment of smoke-induced lung damage.

Xu et al. (2019)	Kenya	Murine macrophage RAW264.7	Anti-inflammatory, antioxidant effects, Phyto chemical profiles	Ethanolic extracts of young leaves, seeds, and roots,	DPPH, ABTS, FRAP Griess reagent protocol	Leaf extracts showed better anti-inflammatory activity. Leaves and roots have higher antioxidant activity.	The positive correlation between flavonoid content and anti-inflammatory and antioxidant activities.
Wisitpongpun et al. (2020)	Thailand	MDA-MB-231 breast cancer cells, K562 human leukemia line, SCC-15 human carcinoma line	Anticancer effects, bioactive compounds	Hexane, ethyl acetate, and ethanol fractionates of leaf powder	LC-ESI-QTOF-MS/MS analysis, cell viability, colony formation, apoptosis, cell-cycle analysis	Inhibition of cancer cell lines, highest by the crude ethyl acetate fraction. Three compounds (7-octanoic acid, oleamide, 1-phenyl-2-pentanol) were identified.	Selective anticancer activity. Oleamide has the highest apoptotic activity.
Luetragoon et al. (2020)	Thailand	Human monocyte-derived macrophages (MDM)	Anti-inflammatory effects, bioactive compounds	Hexane, ethyl acetate fractionates of leaf powder	LC-ESI-QTOF-MS/MS analysis, cytotoxicity assay, qRT-PCR, sandwich ELISA, Western Blot	A decrease in IL-6, TNF-α, IL-1β, and PGE2 in macrophages. Many novel compounds isolated	Leaf ethyl acetate extracts inhibit the inflammatory NF-κB pathway
Potesta et al. (2019)	Italy	Human Jurkat E6-1 lymphoid; THP1 monocytoid cell lines	Anticancer effects	Aqueous extracts of leaves and seeds, boiled and frozen	Cell death, viability assay, proliferation assay, qRT-PCR	Frozen extracts are more toxic than boiled extracts. Small RNAs of MO possibly affected DNA synthesis.	Specific antitumor activity, higher in seeds compared to leaves.
Potesta et al. (2020)	Italy	Human Jurkat E6-1 lymphoid cells, HeLa human cervix epithelioid carcinoma cell line, mononuclear blood cells	Anticancer effects	Microvesicles from seed aqueous extract	Cell death, viability assay, cell cycle, apoptosis assay, JC1 mitochondrial assay	Downregulation of BCL-2, apoptosis of cell lines. Microvesicles can enter human cells and modulate the activity.	Inhibition of cancer cell lines but not monocytes. Microvesicle effects had lower toxicity compared to cruder extracts.
Cirmi et al. (2019)	Italy	SH-SY5Y human neuroblastoma cells	Anticancer effects	Moringin derived from seed glucomoringin	Proliferation, cytotoxicity, apoptosis, cell-cycle assays	Time and dose-dependent inhibition of tumor growth by upregulating caspases and tumor suppressor genes, inhibit NF kB.	Apoptosis of tumor cell line; induce cell-cycle arrest, thereby suppressing growth.
Verma and Singh (2020)	India	Dalton's ascites lymphoma (DL) cell line	Anticancer effects	A methanol extract of MO leaves, along with other sources	Proliferation (MTT), apoptosis, cell-cycle assays	Selective cytotoxic, apoptotic effects on lymphoma cells, mitochondrial-mediated effects.	Good cytostatic activity against tumor cells.

MTT, methyl thiazolyl tetrazolium assay; TBARS, thiobarbituric acid reactive substances; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; ELISA, enzyme linked immunosorbent assay; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, FKBP12 rapamycin-associated protein; iNOS, inducible nitric oxide synthase; COX, cyclo-oxygenase; cMOL, coagulant *M. oleifera* lectin; WSMoL, water soluble lectin from *M. oleifera* seeds

Results

Eighteen studies were selected based on the applied criteria.[17–34] Two independent evaluators performed a randomized evaluation to eliminate timeline and interobserver bias. Table 2 illustrates the information categorized after a comprehensive evaluation. The 18 studies have been reported between 2013 and 2020. Almost every study is recorded from a different country and has representation from every region of the world. Ten studies focused on the antiproliferative (anticancer) effects of the MO extracts. Six studies were on the antioxidant and anti-inflammatory effects. However, most studies had evaluated multiple procedures.

Normal as well as cancer cell lines were cultured in these studies. The normal cell lines included monkey kidney cells, human periodontal stem cells, murine macrophage cell lines, and human dermal fibroblasts. Cancer lines included lung cancer cells, breast cancer, colorectal cancer, pulmonary mucoepidermoid carcinoma, larynx carcinoma, small cell lung cancer, and hepatocellular carcinoma.

All studies employed specifically produced MO extracts. They were derived from leaf, seed, flower, pod, bark, and root (hot treated/boiled aqueous extract, cold treated/frozen aqueous extract, ethanolic extract, fractionated extract, microvesicles). Some studies performed multiple extraction methods from different tree sources to compare their effects.

The studies have employed a wide assortment of laboratory procedures. The most prevalent procedures included cell proliferation/cytotoxicity/viability assays [mainly done by MTT or 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-2H-tetrazolium bromide], oxidation stress assay (TBARS , glutathione), RT-PCR, Western blot, cell-cycle assay, motility assay, gene analysis, TUNEL, and ELISA. These tests were performed to assess the antiproliferative, antioxidant, and anti-inflammatory effects.

The risk of bias assessment is illustrated in Table 3. Overall, the risk of bias was assessed to be low by two independent evaluators.

Table 4 succinctly summarizes the major findings of the selected studies. There are adequate data generated and compiled from these studies to reach clinically significant conclusions regarding the best source of the extracts (leaf, seed seem to be the best sources), and extraction method (heat-treated/boiled aqueous extracts, ethyl acetate fractionation were the most efficacious methods). All studies across the board have reported concurrent effects of MO extracts in cell lines. Studies of cancer cell lines have consistently reported inhibition of cell growth, increased apoptosis, cell-cycle arrest, and cytotoxicity in all cancer cell lines irrespective of the cancer type. These effects were dose-dependent but were seen even at low concentrations. In studies that have included normal as well as cancer cell lines, the selective effects of MO extracts were clear. The extracts did not show inhibition of normal cell lines. Conversely, there was increased proliferation observed in stem cell lines and fibroblasts. There was no cytotoxic or growth suppression except in very high doses. Cancer cell lines were inhibited at much lower concentrations.

Many studies reported the antioxidant and anti-inflammatory effects of MO extracts, especially those employing macrophage cell lines. The macrophage activity was reduced in multiple ways. There was the suppression of pro-inflammatory cytokines like ROS, tumor necrosis factor (TNF)α, interleukin (IL)-1β, nitrous oxide, IL-6 and prostaglandin E2 (PGE2), and upregulation of anti-inflammatory mediators like IL-10 and IκB-α. The nuclear factor kappa B (NFκB) pathway seems to be the major point of MO activity.

Discussion

M. oleifera, the common drumstick tree, grown widely in the tropical climes of the world, has long been known to have significant health benefits. In many countries, the leaves, flowers, and fruit pods are an essential food staple. Powdered extracts are used in Ayurvedic medicine and as for tea. Oil extracted from seeds is used in cooking, as fuel, and in topical formulations and cosmetics. Only in recent years, the medical potential of this wonder plant is being uncovered by scientific methods and is trending in the modern imagination.[1–13]

The role of reactive oxygen metabolites and free radicals in pathological processes, and the antioxidant mechanism, has been generating huge academic interest. Synthetic chemicals used in the treatment of diseases are increasingly implicated in many disorders and exert deleterious effects on body systems. Therefore, safe and natural alternative management is the main focus of present and future drug formulations. Numerous inflammatory and cancerous conditions and lifestyle disorders seem to be free radical and ROS-mediated.[14–16]

The major results of the studies indicate that there are potent antiproliferation and apoptotic activities in cancer cell lines, which was not observed in normal cell lines. The antioxidant activity was seen to be upregulated and inflammatory activity was downregulated, as shown by both marker activity and gene expression. Moreover,

Table 3: Risk assessment of bias across studies (Adapted from: Cochrane Handbook tool. Cochrane Handbook v 5.1.0, March 2011)

Domain	*Risk*	*Review authors' judgment*
Selection bias		
Randomization Allocation concealment	Low/ unclear	These types of studies use cell lines and laboratory procedures. It is unclear if randomization and allocation concealment is applicable
Performance bias		
Blinding of personnel and participants	Low	Automated laboratory procedures of cell lines eliminate subjective errors
Detection bias		
Blinding of assessment	Low	Automated laboratory procedures of cell lines eliminate subjective errors
Attrition bias		
Incomplete outcome data	Low	All data assessed
Reporting bias		
Selective reporting	Low	All data reported
Other bias	Unclear	Insufficient information

Table 4: Qualitative synthesis

Theme/parameter	*Nature of variability*	*Effect of MO extract*	*Report*
Cell line type	Range of cell types, both normal and cancer cells, from animals and humans	Dose-dependent beneficial effects on normal cell lines and cytotoxic effects on cancer lines.	MO extracts seem to possess a beneficial effect on normal cell lines in low doses, while high doses were cytotoxic. In cancer lines, all doses were found to be cytotoxic. Therefore, the ideal concentration of the extracts for therapeutic usage must be determined.
Extract type	Extracts from leaf, seed, pods, flower, bark, roots.	Leaf extracts were the most potent and the seed extracts least.	MO extracts made from leaves seem to be most suitable for therapeutic usage. Aqueous extracts and hexane fractions seem to be safer and effective. Alcoholic extraction and fractionation may be done for specific requirements. This may be done keeping in mind the elevated cytotoxic effect.
	Aqueous extracts, solvents like ethanol, ethyl acetate, chloroform, butanol, hexane, fractionated extracts, moringin.	Alcohol-based fractionation elevated the potency of the studied effect; however, elevated cytotoxicity was also observed.	
	Cold and heat-treated forms, microvesicles	Heat treatment seemed to lower the effect; cold treatment seems to be preferred. Safest seems to be cold aqueous extract. Microvesicle extraction is promising.	Further research on microvesicles required.
Laboratory procedure	Depended on the effect studied. Cytotoxic (MTT) assay, proliferation assay, genetic analysis, inflammatory mediator assays, ELISA, Western Blot, oxidation stress, etc.	All assays were satisfactory in determining the particular effect. Genetic analysis like PCR, TUNEL, Comet, microarray, and transcriptome analysis provided valuable data.	Cytotoxicity assays revealed the destructive effect of MO on cancer cell lines. Proliferation assays showed a positive effect on normal stem cells and other cells. Genetic analysis revealed the upregulation of apoptotic, anti-inflammatory, and antioxidant genes and downregulation of inflammatory genes and oncogenes.
Anticancer effect	Proliferation and cytotoxicity studies, oncogene and tumor suppressor gene analysis	Tumor suppressor (p53, etc) and apoptotic genes (caspase, etc) were upregulated. Antiapoptotic and inflammatory/oxidant effects were downregulated.	MO extracts showed potent anticancer effects. In cancer cells, even prooxidant and inflammatory effects were pronounced. Cell survival and motility reduced.
Antioxidant activity	Oxidation stress assay, nitric oxide, iNOS, COX-2, ABTS	Inhibition of reactive oxygen species. Nitrite formation reduced, iNOS downregulated. But the potent effect was seen on normal cell lines and not cancer lines.	Potent antioxidant effect of MO extracts. The opposite effect was seen in some trials of cancer lines.
Anti-inflammatory activity	Cell mediator assays, inflammatory gene analysis	Pro-inflammatory mediators like PGE2, IL-1α, IL-6, IL-8, TNFα, RelA (NFKB) reduced. Anti-inflammatory mediators like IL-10, iκBα	Potent anti-inflammatory effects in genetic level, upregulation of anti-inflammatory genes/products, and downregulation of pro-inflammatory genes/products.
Other effects	Neurogenesis, stem cell proliferation, wound healing	Beneficial effects on wound healing, nerve regeneration, stem cell proliferation.	Positive effect on normal cell lines, indicating a role in regeneration, healing.

these effects seemed to be dose-dependent. Lower concentrations showed maximum beneficial effects, while higher concentrations produced comparable cytotoxicity even in normal cell lines.[17–34]

MO extracts are not cytotoxic to normal human cell lines. A 2018 neurogenesis study revealed that moringin [4-(α-L-rhamnosyloxy)-benzyl isothiocyanate], an MO derivative, exerted neuro-differentiation among periodontal ligament stem cells. Therefore, there is evidence that this derivative might selectively be cytotoxic to tumor cells in low doses.[29] This activity was further confirmed in a 2019 study in which cancer cell lines were inhibited by inducing cell-cycle arrest and apoptosis.[25]

There is considerable variation in the effects produced by the different sources of MO extracts. Most studies agree that leaf extracts achieved the most potent beneficial effects, while seed extracts were the weakest.[20,26,27] Pods, flower, and bark extracts showed intermediate results. Even within the type of extracts, the method of extraction proved to be significant. Among aqueous extracts, heat-treated extracts exhibited less potency compared

to cold-treated extracts. This could indicate that certain beneficial components might be inactivated by heat. A 2019 analysis of macrophage cell lines reported that root extracts were as effective as the other sources of the plant. Their anti-inflammatory and antioxidant activities seemed to correlate with the flavonoid content of the extracts. A 2020 study found many specific compounds and in particular reported oleamide as having the highest antiapoptotic activity against tumor cell lines.[20,21,30–32]

Ethanolic extracts were more potent than the aqueous extracts, possibly due to the preferential concentration or isolation of active components during the extraction process. Further, fractionated extracts were incorporated in some studies. For extraction, solvents like ethanol, hexane, dichloromethane, ethyl acetate, butanol, and chloroform were used. Results indicate that the ethyl acetate fraction might demonstrate the most potent beneficial activity. Higher potency was also demonstrated by cold aqueous leaf extracts, ethanol extract, and chloroform fraction. In one study, the hexane fraction showed the least cytotoxicity compared to the other extract types.[19,33,34]

The above-mentioned finding corresponds with the earlier finding regarding dose-dependent potency and cytotoxicity. Very high doses and prolonged fractionation demonstrated higher results but significantly also produced deleterious effects in normal cell lines.

Therefore, further research must take into account the optimal nature and the dosage of the active compounds in these extracts.

Cell proliferation, cytotoxicity, and survival assays performed using these extracts produced significant results. Increased p53 activity, caspase upregulation, increased apoptosis, G2-M cell-cycle arrest, etc., of neoplastic cell lines were reported in many studies. The effects mainly pointed to the upregulation of tumor suppressor genes and downregulation of oncogenes. Notably, normal cell lines like stem cell and dermal fibroblast studies showed increased proliferation especially in low concentrations.[17–34]

As regards antioxidant and anti-inflammatory activities, results indicate that MO extracts could be a potential game changer. Most studies consistently report the effects of MO on critical pathways of inflammation and free radical dynamics. Inhibition of inflammatory mediators and oxidants like ROS, TNFα, and IL-1β were reported. Nitrite formation and PGE2 were inhibited but anti-inflammatory IL-10 and Iκ-B-α were increased. Nitrous oxide and IL-6 were also inhibited. Downregulation of iNOS and COX-2 suppressed IκBα degradation, attenuated NFκB expression was also reported. Notably, reduced RelA (a subunit of the NFκB pathway) was found in a study. Many studies imply that one of the anti-inflammatory mechanisms of MO is routed via the vital NFκB pathway.[22,30–34]

Other significant effects include their positive influence on neurogenesis, wound healing, adipogenesis and osteogenesis, and reducing smoke-induced lung damage.[20,29,32] There has been further research on the exact components of the extracts which exert the beneficial actions.

Recently small RNAs and microRNAs of phytochemicals are increasingly being targeted for their anticancer and anti-inflammatory effects. A 2019 report suggested that these RNAs alter the DNA synthesis in cancer cell lines. A microvesicle extraction study by the same team revealed that plant microvesicles can enter the human cells and exert anti-inflammatory and antioxidant activities. The cytotoxic potential of microvesicles was lesser compared to the cruder extracts, indicating that more research may prove valuable in this aspect. Recent Indian research postulated that the cytotoxic and apoptotic effects of aqueous extracts of MO might be mediated by mitochondrial degradation by the production of reactive oxygen species.[23,24,26]

Recently there has been an explosion of research on phytochemicals including MO extracts in their health effects. Vongsak et al. used 70% ethanolic extraction of MO leaf and found that phytochemicals especially cryptochlorogenic acid (0.05% w/w) and isoquercetin (0.09% w/w) seem to be the major antioxidant components. Another study employed 80% methanolic and 70% ethanolic extraction of freeze-dried leaves. Interestingly comparison of leaves from different agroclimatic conditions revealed that the highest antioxidant effects were noted in Indian varieties. In this study, the active components were found to be quercetin and kaempferol.[38,39]

Basic phytochemical analysis of ethanolic flower extracts revealed compounds like tannins, cardiac glycosides, flavonoids, and alkaloids. Total phenolic content was 19.31 mg/g of gallic acid equivalent. The anti-inflammatory effect was claimed to be comparable to diclofenac. Santos et al. found that ethanolic extract of leaf tissue is much more potent than saline extract in antioxidant (radical scavenging) activity.[40,41] Further, vitamins and their precursors, e.g., niacin, ascorbic acid, beta carotene, and tocopherol, have been isolated. This might explain the anti-inflammatory and antioxidative effects.[42,43]

All the above-mentioned studies indicate that leaf extracts might be the most beneficial in therapeutic use. Further, the effect of tender (immature) and mature leaves were evaluated by a study that reported that both stages of leaves possessed good antioxidant activities. However, a Thai study found that aqueous extracts had higher antioxidant capacities and total phenolic content than ethanolic extracts, which was a departure from most of the observed studies. They also found that the antioxidant activity was more regarding quenching of ABTS [2,2′-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)] cation decolorization than the reducing power and quenching of DPPH.[44,45]

Extensive reviews and animal studies of the various applications of *M. oleifera* extracts have been published. The phytosterols found in MO extracts include phytosterols, glucosinolates, caffeoylquinic acid, and glycosides. They credit the anticancer effect to components like hexadecanoic acid ethyl ester. The antioxidant properties of MO extracts were due to the suppression of malondialdehyde and an increase in ascorbic acid, superoxide dismutase, and glutathione peroxidase. Their cytokine modulation and anti-inflammatory effects on macrophages via multiple signaling pathways through effects on NF-κB, IκBα, and TNF-α have been documented. They may also be effective in treating immune disorders.[46–49]

A qualitative synthesis of the studies generated valuable information (Table 4). Based on the results and analysis of the studies, the applications of *M. oleifera* extracts could be summarized as follows: extracts of leaves of *M. oleifera* were the most effective. Other plant parts had beneficial effects, though not comparable to leaves. Aqueous and ethanolic extraction at room temperature seemed to be the best method. Fractionation has been reported to generate higher potency but the adverse effects were also magnified. Microvesicle extraction could hold great potential for future research. The effects were dose-dependent. Cytotoxicity has been reported in normal cell lines in higher dosages. Cytotoxicity was reported in cancer cell lines even at low concentrations. Paradoxically in cancer cells even prooxidant properties were observed. MO extracts had significant anti-inflammatory,

antiproliferative, and antioxidant properties. Many of the effects were detected at the molecular and genetic levels (RNAs and mitochondrial).

Therefore, there is extensive evidence of the beneficial properties of MO extracts. There is a need for standardization and quality control in the manufacture of these extracts. Chromatographic fingerprinting can help in the assessment of the quality of these products. Karthivashan et al. using HPLC–DAD–ESI–MS (High performance liquid chromatography [HPLC] with diode array detection, electrospray ionization, and mass spectroscopy) found that a 90% ethanolic extract had the most effective extraction of bioactive components. Apart from the previously identified kaempferol and quercetin, new compounds multiflorin-B and apigenin were found in the leaf extracts. Makita et al. compared *M. oleifera* and *Moringa ovalifolia* and found superior flavonoid content in the former, especially a compound known as vicenin-2, an antidiabetic and antioxidant molecule. They further opined that genetic and environmental influences play a significant role in determining the flavonoid profile of the specimens. Chinese researchers follow the Traditional Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (v 2004A) for quality testing of samples. Xu et al. analyzed leaf extracts using ultra-high-performance liquid chromatography (UPLC) and found twelve common peaks, which they determined to be the characteristic fingerprint. Xu et al. found differences in the phytochemical composition between leaf, root, and seed extracts. The main chemicals found with strong activity were kaempferol 3-O-glucoside, kaempferol 3-O-rutinoside, quercetin 3-O-(6″-malonyl-glucoside), and a quercetin derivative. Zhu et al. in an analysis of MO seeds derived from 11 geographic regions in China, India, and Myanmar found that eleven basic chromatographic peaks were concurrent. The optimal extraction method seemed to be a 24-hour soak extraction followed by 30-minute ultrasonic extraction using 50% methanol. They concluded that HPLC-DAD was a simple and efficient method to determine the quality of samples. Further research would enhance the quality of extraction and optimize therapeutic benefits.[20,50–54]

Conclusion

M. oleifera extracts possess significant health benefits. They possess anti-inflammatory, antiproliferative, and antioxidant effects. MO leaves are the best sources of extracts, especially under aqueous and alcoholic extraction methods. They are cytotoxic to cancer cell lines and protective in normal cell lines; their effects being dose-dependent. Further studies are necessary for quality control of production and therapeutic applications.

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