

Moringa oleifera Lamarck (1785), Moringaceae and Cancer I: A Systematic and Comprehensive Review of 24 Years of Research

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Abstract

This review was aimed to assess the anti-cancer properties of *Moringa oleifera* Lamarck. (Moringaceae: MO) reported from 1998 till 10 November 2021. A total 71 PubMed relevant papers were discussed here. Among all parts of MO which used to assess antitumor activities, the leaves (52%), seeds (22%), pods (7%), and phytochemicals (7%; like moringin and its congeners) would be considered as a source of putative *phyto-oncolytics* or *phyto-oncostatics*. The partitioning of secondary metabolites with pharmacological value in source (leaf) and sink (roots, flowers, pods, callus, and fruits) organs of MO dictates the best choice of the solvents for their extraction. The polar: water (29%) > ethanol (17%) > methanol (13%) > hydro-alcohol (11%); intermediate polar: dichloromethane (4%); and nonpolar: n-hexane = ethyl acetate (7%) > chloroform (3% of studies) solvents have been employed for extractions among studies. The human colorectal cancer, leukemia, non-small cell adenocarcinoma, and breast cancer consisted 22, 14, 10, and 8% of screened studies, respectively and the rest of tumors consisted less than 5% of studies. The *in vitro* (51%), *in vivo* chemically induced model (21%), and tumor graft models (8%) were reported and there were no clinical trials among studies. Totally, from 118 cell lines used, healthy cell lines (control; $n = 19$), MCF-7 ($n = 12$), HepG2 ($n = 12$), and HeLa ($n = 6$) consisted top list amongst studies. From 76 anti-cancer portals curated amongst studies, induction of apoptosis ($n = 29$), anti-proliferation ($n = 17$), anti-angiogenesis ($n = 8$), and DNA/RNA fragmentation ($n = 6$) were the main antitumor portals and the cytotoxicity and anti-inflammation may be considered as minor ones. To sum up, the rational extraction and purification of MO, phytochemistry, and computational and experimental pharmacology of various extracts of MO should be pursued to decipher *phyto-oncolytic* and/or *phyto-oncostatic* drug-like phytochemicals suitable to be employed in clinical trials.

Keywords: Moringa- antitumor- apoptosis- human colorectal cancer- leukemia- non-small cell adenocarcinoma- breast cancer

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Introduction

Economics of cancer and botanicals

Cancer stands amongst the top-ranking mortality causes across the world, although spectacular onco-diagnostic and onco-therapeutic advances have been occurred in previous decades. At the present time, the imprisonment of humankind in a heavily polluted environment replete with tumorigenic agents has posed a radical challenge to the human health recognized as “*cancer boom*”. This critical issue has especially placed a tremendous strain upon those countries confronted by inadequate medical infrastructure and limited access to modern onco-therapeutic arsenals such as chemo- and

radio-therapies.

Numerous institutes and academia have put forth a great deal of effort towards developing effective oncotherapeutics, but, by contrast, stiff resistance has been gained by different tumors in response to the available chemo-therapeutics and radio-therapeutics.

There exists a long list consisting of a vast spectrum of modern botanical-based anti-cancer drugs which are also referred to as botanical onco-therapeutics [1]. It is considerably needed to labor this noteworthy point that a huge number of anti-cancer phytochemistry or

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formulations never reach to clinical trial setting due to many challenges and barriers such as the lack of systematic integration in preclinical studies and financial support [2]. Along with all is known and reported, miscellaneous undiscovered and must-be-explored aspects exist as to the potential biological targets which could be targeted and help us battle against cancer.

All considered, an array of pharmacodynamics has been summarized in Figure 1 proposed for anti-cancer botanicals in seminal reviews [3, 4].

Nowadays, developing countries have risen to the challenge of low economic income as well as unstable market of anti-cancer drugs which have been posed by complex problems including undeveloped infrastructures for the development of antitumor drugs, restricted access to the knowledge should be replaced by technology urgently required to develop pharmaceutical products by means of advanced bioreactors, resilient political and economic import systems, and tight restrictions and hard sanctions imposed upon the import of required products. To sum up, tremendous efforts have been focused on seeking antitumor botanicals to come up with a solution in the face of onco-therapeutic challenges (Figure 1).

Moringa oleifera (Lamarck, 1785): An ultrashort overview

Moringa oleifera (Lamarck, 1785; MO; Figure 2) is a perennial deciduous tropical tree and medicinal plant which is taxonomically classified in the order Brassicales, family Moringaceae and genus *Moringa* native to West Africa and the Indian subcontinent. This immensely valuable tree is widely planted in India due to its profound significance in pharmaceutical industry. Likewise, this plant has been imported and planted in many countries such as Iran in which it is locally referred to as *oil tamarix* or *castle tamarix*. Globally, MO is known under various aliases *Viz*, *Miracle Tree*, *Horse Radish Tree* and *Drumstick Tree* in English, *Ben Oil Tree*, *Benzoil Tree*, the *Murungai* or *Kelor* in Malaysian, *Zogale* and *Gerged* in Nigerian, *Shigon* in the Shushruta Sanhita of India, *Sajiwani*, *Sajna*, *Soanjna* and *Munga* across India. The plant is also known *Shobhanjana* (Sanskrit), *Saragvo* (Gujarati), *Sajna* or *Sajina* (Bengali), *Munanga* (Telugu) and *Murungai* (Tamil) in various Indian linguistic variants.

Adopting a pharmacognostic perspective through looking askance at medicinal properties found in almost all parts of MO (for a review see [5]), it could serve a therapeutic role against various pathological circumstances including asthma, allergy, pain, fever, ulcers, neurological disorders, cardiovascular diseases, diabetes mellitus, hepatocellular disorders, inflammatory conditions, infertility, infectious diseases, and more specifically neoplastic conditions (for a review see [6-8]).

This plant contains a diverse array of functional bioactive components including vitamin A, vitamin C, proteins, alkaloids, quinines, saponins, flavonoids, tannins, steroids, glycosides, fixed oils and fats, and phytochemicals like niazinin A, niazinin B, niazimicin A, and niaziminin B (for a review see [6, 9, 10]). Adopting an approach in the light of orthodox medicine, this systematic review was designed to highlight anti-cancer

properties of MO.

Methods

Search strategy

We extensively launched a search in PubMed database until November, 11, 2021 to find papers published with search string: “(Moringa) AND (cancer)” on the basis of PRISMA guidelines [11] as illustrated in Figure 3. Studies were carefully screened and eligible ones were selected based upon the following inclusion criteria: (1) studies published in English; (2) studies aimed at reporting the anti-cancer effect (s) of MO; and (3) studies carried out at the level of human subjects or animal model. We excluded papers focused on the green synthesis of phyto-nanoparticles using MO and review articles; however, their references were scrutinized to seek relevant works.

Eligibility criteria

All included papers were subject to data extraction respecting the following information: (1) the part of MO used, (2) the employed formulation, (3) the animal or tumor model used and (4) the mechanism or targeted pathway.

Results

General view

I found 129 articles in accordance with the initial search script (*vide supra*). To begin with, based upon the close examination of titles and abstracts, 21 articles were excluded due to being review articles, not including MO, or lacking the evaluation of anti-cancer effects of MO.

In addition, after full text reading of the articles, 20 ineligible ones were excluded due to being focused on the green synthesis of nano-compounds using various types of MO extracts and-or not being exactly relevant to anti-cancer effects of MO. Finally, 71 eligible articles included for this first systematic review on MO and cancer plus 9 manually-curated records included by checking the reference lists of review articles and eligible studies were selected (Figure 3).

All selected studies are summarized in Tables 1. To conduct a comprehensive review, here, we have categorized the articles according to the following criteria (1) the type of MO-derived formulation, (2) the cell lines or animal models used to investigate anti-cancer effect of MO, (3) the targeted pathway or endpoints reported in studies. Meta-analysis could not be carried out owing to overt heterogeneity in tumor models and outcomes in the selected studies, however descriptive statistics has been pursued to discover the climate of papers have been selected (*vide infra*).

Dermatological cancers

Tender pod or the fruits of MO has a special place in Thai cuisine. Previous studies have demonstrated that MO pod extract can serve a bifunctional role in both displaying antioxidative properties and inhibiting skin

papillomagenesis in mice as well [12]. In this respect, niazimicin has been isolated from ethanol extract of MO seeds and it exerted significant antitumor effect upon the two-stage carcinogenesis in mouse skin using 7,12-dimethylbenz (a) anthracene (DMBA) as the initiator and 12-O-tetradecanoyl-phorbol-13-acetate as the tumor promoter [12].

A hydro-alcoholic extract of whole MO brought about modulatory effects upon phase I (cytochrome b5 and cytochrome P450) and phase II (glutathione-S-transferase) drug metabolizing enzymes, anti-oxidant enzymes, glutathione content and lipid peroxidation within the liver tissues of female Swiss albino mice [13]. Additionally, it possessed chemopreventive efficacy in a two stage model of DMBA-induced skin papillomagenesis [13].

The anti-neoplastic properties of the leaves, root, cortex, and flowers of MO on murine B16F10 melanoma cells, brought about a significantly decreased growth and proliferation rate of tumor cells, cell cycle arrest, the increased expression of p21WAF1/Cip1, p53, and p27Kip1 proteins, and induction of differentiation [14]. The soluble cold distilled water extract (4°C; 300 µg/mL) from MO, greatly induced apoptosis, inhibited tumor cell growth, and lowered the level of internal reactive oxygen species (ROS) in A431 human epidermoid carcinoma [15]. In this regard, the seeds of MO contain carbohydrate-binding proteins, namely lectins, being able to exert strong cytotoxic effects against tumor cells. The cytotoxic effect of coagulant MO lectin (cMoL) upon B16-F10 murine melanoma cells has been previously reported [16].

In another study, the extract of MO leaves was fractionated using different solvents to figure out which fraction is the most effective one having anti-proliferative effect on melanoma cells [17]. In this line, they concluded that the phenolic-rich fraction exerted significant *in vitro* antitumor effects, mediated by mitochondrial ROS, on melanoma cells through both caspase-dependent (activation of caspase-3/7 and caspase-9) and caspase-independent (activation, and translocation of apoptosis-inducing factor into the nucleus) apoptosis pathways.

With regards to dermatological cancers, whole MO plant, its various parts, its cMoL, and even its phytochemical, namely niazimicin, happened to establish chemotherapeutic and chemopreventive impacts on murine B16F10 melanoma cells and skin papillomagenesis [12-14, 16].

Do and colleagues (2020) reported the anti-proliferation mediated by apoptosis of an aqueous extract of MO on human melanoma cell lines through a battery of tests focused on apoptosis [17]. Additionally, in a mechanistic study, Do and colleagues (2021) investigated the anti-proliferative activity activities of various extracts of MO against melanoma cells and normal cells and they decipher caspase-dependent and caspase-independent pathways which took part inter alia [18]. Finally, authors concluded that among all extracts and fractions, the phenolic-rich fraction exerted noteworthy anticancer effects on melanoma cells *in vitro*.

Luetragoon et al. (2021) investigated the anti-cancer

effect of leaf extracts and their fractions, 3-hydroxy- β -ionone of MO on squamous cell carcinoma, SCC15 cell line. They found that MO extracts and 3-hydroxy- β -ionone significantly exhibited antiproliferative effects on SCC15. Moreover, they showed the inhibitory effects of aforementioned formulation on colony formation and cell migration of SCC15 [19].

To sum up, eight papers focused on the useful effects of various MO extract on four cancer cell lines including human melanoma, human epidermoid carcinoma, human keratin-forming tumor, and squamous cell carcinoma.

Gastrointestinal cancers

Three cold, hot, and ethanol extracts were prepared from the leaves of MO which caused death in the majority of hepatocellular carcinoma cells [20]. In this regard, hot water and ethanol extracts were more effective active against aforementioned cancer cells. The chemopreventive effect of boiled freeze-dried MO on azoxymethane (AOM)-initiated and dextran sodium sulfate (DSS)-promoted colon carcinogenesis in mice was demonstrated by suppressive effects induced in a colitis-associated colon carcinogenesis model [21]. The consumption of MO with the aim of providing an alternative/complementary therapy for cancer treatment, is on the rise in Thailand [22]. The inhibitory effect of the aqueous and ethanol extracts from MO leaves on the proliferation of colon cancer cells has been reported [22]. In line with this, two extracts were checked for their non-mutagenicity through safety test, Ames test, and antiproliferative effect on 3 types of colon cancer cell lines including HCT15, SW48 and SW480 showed both a dose-dependent and exposure time-dependent manner. To be more specific, the antiproliferative effect produced by the ethanol extract of MO appeared to be more powerful in comparison with that of the aqueous extract on all tested cell lines. In addition, SW48 was found to be the most sensitive cell line [22].

Hepatoprotective and antioxidative effects of MO against DMBA-induced hepatocellular damage has been reported in mice [23]. In this context, single oral administration of DMBA to mice resulted in significantly plummeted levels of xenobiotic enzymes such as cytochrome P450 and b5 which mediate hepatic carcinogenesis [23]. An extensive use of edible plants such as MO can be traced back to a long time ago in Thai culture so as to heal ulcers and the symptoms of hepatic disease. In this regard, buffered extracts from the fruits of MO showed to have anti-hepatitis B virus activity and also a mild cytotoxic effect on the hepatocellular carcinoma (HepG2) cells [24].

The effect of MO leaf extract alone and in combination with cisplatin on the survival of cultured human pancreatic cancer cells inhibited the growth of pancreatic cell lines including Panc-1, p34, and COLO 357 through deregulation of NF- κ B signaling pathway [25]. Moreover, MO leaf extract synergistically enhanced the cytotoxic effect of cisplatin on Panc-1 cells [25].

Two coagulant lectins including cMoL and water soluble lectin from MO seeds (WSMoL), extracted from the seeds of MO, have been used worldwide to

Table 1. A Historical and Systematic Review on the Antitumor Effects of *Moringa oleifera* Lamarck

Reference	Formulation	Cell line/Animal model	Pathway	Outcome
Murakami et al., 1998 [26]	Leaf (niaziminin derived from methanol extract)	The activation of tumor-promoter-induced Epstein-Barr virus (EBV) in Raji cells as an EBV-containing Burkitt's lymphoma cell line	The inhibition of EBV activation	Promoting antitumor effect
Guevara et al., 1999 [12]	Seed (ethanolic extract)	Two-stage carcinogenesis in mouse skin initiated by 7,12-dimethylbenz (a) anthracene (DMBA) and promoted by 12-O-tetradecanoyl-phorbol-13-acetate	The inhibition of EBV-early antigen activation	Promoting antitumor effect and chemo-prevention of carcinogenesis
Bharali et al., 2003 [13]	Whole plant (ethanol extract)	DMBA-induced papillomagenesis in mouse skin	Increased activity of glutathione-S-transferase (GST), cytochrome p450 (CYP P450), cytochrome b5 (CYP b5), catalase, glutathione reductase (GR), acid soluble sulfhydryl content (-SH), glutathione peroxidase (GPx), and significantly reduced activity of hepatic malondialdehyde	Chemo-prevention against chemical carcinogenesis
Khalafalla et al., 2010 [20]	Leaf (cold water, hot water, and 80% (v/v) ethanol extracts)	Acute myeloblastic leukemia (AML), acute lymphoblastic leukemia (ALL) cells, and HpG2 human hepatocellular carcinoma (HCC) cells	Not access to data	Antitumor activity
Promkum et al., 2010 [27]	Pod (boiled; bMO)	Mitomycin C- and DMBA-induced clastogenicity in mouse	Altered number of micronucleated peripheral reticulocytes	Anti-clastogenic potential
Brunelli et al., 2010 [28]	Seed (glucomoringin-derived isothiocyanate)	Xenograft tumor model of RPMI8226 human myeloma cancer cells in severe combined immunodeficiency disease mice	Induced apoptosis through a caspase-dependent pathway, NF- κ B inhibition	The reduced growth of myeloma tumor and antitumor activity
Budda et al., 2011 [21]	Pod (boiled; bMO)	Azoxymethane-initiated and dextran sodium sulfate-promoted colon carcinogenesis in mice	Significantly decreased PCNA index and expression of iNOS and COX-2 proteins	Chemo-prevention, anti-inflammation
Paliwal et al., 2011 [29]	Pod (hydro-ethanolic extract)	DMBA-induced renal carcinogenesis in Swiss albino mice	Normalization of renal oxidative stress markers including lipid peroxidation (LPO), superoxide dismutase (SOD) and catalase (CAT)	Anti-nephrotoxic effect, anti-oxidant
Sreelatha et al., 2011 [30]	Leaf (boiled/cooled aqueous extract)	Human KERATIN-forming tumor (KB) cell line	DNA Fragmentation, modulation of redox-sensitive mechanism, ROS production	Anti-proliferation, potent induction of apoptosis
Pamok et al., 2012 [22]	Leaf (hydro-ethanolic extract)	HCT15, SW48 and SW480 colon cancer cell lines	Not free access to fulltext	Anti-proliferative
Sharma et al., 2012 [23]	Pod (hydro-ethanolic extract)	DMBA-induced hepatic carcinogenesis in mice	Modulation cytochrome p450 (CYP P450) and cytochrome b5 (CYP b5), decreased level of glutathione (GSH), and glutathione-S-transferase (GST)	The induction of apoptosis, chemo-prevention
Waiyaput et al., 2012 [24]	Leaf (hydro-alcoholic extract)	HepG2 human liver cancer cells, COS-7 cells	Inhibited and significantly decreased hepatitis b virus (HBV) cccDNA in transiently transfected HepG2 cells	Cytotoxicity and anti-HBV activity
Gismondi et al., 2013 [14]	Leaf, root, cortex, flower, seeds (boiled aqueous extract)	B16-F10 melanoma cells	The scavenging of free radicals, the enhancement of p53, p21 WAF1/Cip1 and p27 Kip1 protein levels	Anti-oxidant, anti-proliferative and differentiative effects
Berkovich et al., 2013 [25]	Leaf (boiled aqueous extract)	Panc-1 and COLO-357 human pancreatic cancer cell lines	The inhibition of growth and NF- κ B signaling pathway, synergistic effect on chemotherapeutic efficacy of cisplatin	Cytotoxicity, chemotherapeutic effect on chemo-resistant tumors
Tiloke et al., 2013 [31]	Leaf (aqueous extract)	A549 lung cancer cells	Significantly increased expression of p53, increased activity of caspase-9, and caspase-3/7; enhanced expression of Smac/DIABLO, and induced cleavage and activation of PARP-1, reduced mRNA and protein expression of Nrf2, and reduced glutathione (GSH)	Anti-proliferation
Araújo et al., 2013 [32]	Seed (aqueous extract; lectins including cMoL and WSMoL)	NCI-H292, HT-29, HEP-2 / Male Balb/c mouse model of carrageenan-induced pleurisy	Exhibition of anti-inflammatory activity by means of reducing the production of nitric oxide, TNF- α , and IL-1 β along with decreasing myeloperoxidase activity	Cytotoxicity, immuno-suppression, and anti-inflammatory activity

Continued Table 1.

Reference	Formulation	Cell line/Animal model	Pathway	Outcome
Ghosh, 2014 [33]	Leaf (methanol extract)	MCF 7 epithelial breast cancer cell line and MDA-MB-231 mesenchymal breast cancer cell line	Decreased adhesive capacity of MCF-7 and MDA-MB-231 cells	Anti-proliferation, cytotoxicity
Charoensin, 2014 [34]	Leaf (methanol and dichloromethane extracts)	HepG2 human hepatocellular carcinoma cells, Caco-2 human colorectal adenocarcinoma cells, MCF-7 human breast adenocarcinoma cells, and human dermal fibroblast	Quinone reductase induction assay, cytotoxicity	Antioxidative, anti-proliferation, and chemo-prevention
Jafarain et al., 2014 [35]	Callus and Leaf (ethanol-water (70-30) extracts)	Hela human cervix carcinoma cell line	Decreased viability of Hela cells induced by the antioxidative activity	Induced cytotoxicity
Akanni et al., 2014 [36]	Leaf (ethanol extract)	Benzene-induced leukemia bearing rats	Potent antioxidative activity mediated through decreased malondialdehyde and enhanced glutathione (GSH)	Antioxidative, chemopreventive and anti-leukemic effect
Jung et al., 2014 [15]	Leaf (soluble aqueous extract)	MCF-7 breast adenocarcinoma cell line, A431 human epidermoid carcinoma cell line, HT1080 human fibrosarcoma cell line, and COS-7 monkey fibroblast/ fibroblast-like cells	Upregulation of heat shock proteins including HSPA8, HSPA1B, HSP90AB1, and HSPD1; downregulation of Akt, p-IkB, NF-κB, p-Erk, β-catenin, and cyclin D1	Anti-proliferation, cytotoxicity
Leelawat and Leelawat, 2014 [37]	Leaf and seed extracts	RMCCA1 and KKU100 human hilar cholangiocarcinoma cell lines	Decreased mitochondrial membrane potential and increased production of ROS	Induced cytotoxicity
Elsayed et al., 2015 [38]	Seed (essential oil extracted by cold pressing)	HeLa human cervix carcinoma cells, HepG2 human hepatocellular carcinoma cells, MCF-7 human breast adenocarcinoma cells, CACO-2 human colorectal adenocarcinoma cells and L929 mouse fibroblast cell line	Cytotoxicity	Potent cytotoxic activities
Al-Asmari [39] et al., 2015	Leaf, bark, and seeds (soluble ethanol extracts)	MDA-MB-231 human epithelial breast cancer cell line and HCT-8 human ileocaecal adenocarcinoma cell line	Induced cell cycle arrest at G2/M phase	Anti-cancer properties and induction of apoptosis (leaf and bark extracts)
Diab et al., 2015 [40]	Leaf (50% ethanol extract)	A549 lung adenocarcinoma cell line, PC-3 prostate adenocarcinoma cell line, T47D and MCF-7 breast cancer cell lines, HCT-16 and Colo-205 colon cancer cell lines, and THP-1, HL-60 and K562 leukemic cell lines	Cell cycle arrest at G0/G1 phase, increased DNA fragmentation	Anti-proliferative activity
Jung et al., 2015 [41]	Leaf (cold soluble aqueous extract)	A549 human non-small cell lung cancer, HepG2 human hepatocellular carcinoma cells, and male immune-deficient nude mice	DNA strand breaks	Inhibitory effect on tumor progression and clonogenicity of cancer cells, apoptosis induction, and anti-proliferation
Krishnamurthy et al., 2015 [42]	Leaf (n-hexane, chloroform, ethyl acetate, methanol extracts and 15 fractions (F1 to F15) of ethyl acetate extract)	Hep-2 hepatocellular carcinoma cell line and Dalton's lymphoma ascites model in mice	Potential cytotoxic effect on Hep-2 cell lines, downregulation of ERK1/2 phosphorylation	Antitumor activity, cytotoxicity
Fernandes et al., 2016 [43]	Leaf and flower (aqueous extracts)	MCF-7 human breast adenocarcinoma cells	Angiogenesis via chorioallantoic membrane assay, cytotoxicity	Anti-proliferation, cytotoxicity, anti-inflammation; proangiogenesis in normal tissue
Maiyo et al., 2016 [44]	Seed and leaves (crude extract)	HepG2 hepatocellular carcinoma cell line, Caco-2 human colorectal adenocarcinoma cell line, and HEK293 non-cancer cell line	Viability, cytotoxicity	Cytotoxicity and pro-apoptotic effect upon both cancer and non-cancer cell lines, antitumor effect
Rajan et al., 2016 [45]	Seed (glycosylated isothiocyanate moringin)	Human astrocytoma: grade IV CCF-STTG1 cells	The activation of p53 and Bax, Bcl-2 inhibition, modulation of Nrf2 transcription factor and its upstream regulator CK2-alpha expressions along with DNA and RNA fragmentation	The induction of oxidative stress-mediated apoptosis, antitumor activity

Continued Table 1.

Reference	Formulation	Cell line/Animal model	Pathway	Outcome
Tiloke et al., 2016 [46]	Leaf (boiled aqueous soluble extract)	SNO esophageal cancer cell line	Enhanced lipid peroxidation and DNA fragmentation, increased externalization of phosphatidylserine (PS), enhanced activities of caspase-9 caspase-3/7; and increased expression of Smac/DIABLO protein and cleavage of PARP-1	Anti-proliferation, apoptosis induction
Michl et al., 2016 [47]	Seed (glycosylated isothiocyanate moringin)	HeLa cells human cervix cancer cell line, the interleukin-3 (IL-3)-dependent mouse pro-B cell line Ba/F3, the Ba/F3tet-on-1*6 cell line	Inhibition of TNF-induced NF- κ B and IFN α -induced STAT1 and STAT2 activities	Chemo-prevention
Förster et al., 2016 [48]	Leaf (methanolic extract (α -4-rhamnopyranosyloxy-benzyl glucosinolate (Rhamno-Benzyl-GS) and acetyl- α -4-rhamnopyranosyloxy-benzyl glucosinolate Isomer III (Ac-Isomer-GS III))	HepG2 hepatocellular carcinoma cells and V79-MZ Chinese hamster lung cells	The activation of the Nrf2 pathway	Induction of hepatic detoxifying enzymes; antitumor activity
Madi et al., 2016 [49]	Leaf (soluble boiled aqueous extract)	A549 adenocarcinomic human alveolar basal epithelial cells, HepG2 human hepatocellular carcinoma cells, Caco2 human colorectal carcinoma cells, Hek293 human embryonic kidney, and Jurkat cells	Decreased mitochondrial membrane potential, and ATP levels, followed by increased ROS, caspase activation, expression of pro-apoptotic proteins including p53, SMAC/Diablo, AIF, and PARP-1 cleavage	Apoptosis induction, cytotoxicity, and antitumor activity
Cuellar-Núñez et al., 2017 [50]	Leaf (powder)	Azoxymethane/dextran sodium sulfate-induced colorectal carcinogenesis in CD-1 (ICR) mice	Decreased activity of harmful fecal enzymes including β -glucosidase, β -glucuronidase, tryptophanase, and urease	Chemo-protective, diminished colonic lesions, and decreased activity of fecal harmful enzymes
Sadek et al., 2017 [51]	Leaf (ethanol extract)	Diethyl nitrosamine-induced HCC in rat	Decreased expression of Bcl-2, Bcl-x1 and b-arrestin-2, upregulated expression of Bax and caspase-3	Chemo-prophylactic efficacy (through implementation of antioxidant activity and actuation of apoptosis)
Tragupakseerojn et al., 2017 [52]	Leaf (ethanol extract)	HCT116 human colon cancer cell line	Downregulation of ERK1/2 phosphorylation	Anti-proliferation
Leelawat and Leelawat, 2017 [53]	Fruit pulp and seed (ethanol extract)	RMCCA1 human cholangiocarcinoma (CCA) cell line	The activation of protein kinase B (Akt), increased expression level of mitogen-activated protein kinase (MAPK) pathways (including ERK1/2 and p38 MAPK), increased activity of pro-apoptotic molecules including caspase-3, poly (ADP-ribose) polymerase, checkpoint kinase 2 and tumor protein 53	Anti-proliferation
Adebayo et al., 2017 [54]	Seed (crude water extract, n-hexane and dichloromethane fractions)	MCF7 human breast cancer cell line, MCF, 10A normal human mammary epithelial cells	Not reported	Anti-proliferation
de Andrade Luz et al., 2017 [16]	Seed (coagulant lectins)	B16-F10 murine melanoma cells	Mitochondrial ROS production, activation of caspases 3, 8 and 9	Cytotoxicity and induced apoptosis
Giacoppo et al., 2017 [55]	4- (α -L rhamnopyranosyloxy) benzyl ITC (moringin) complexed with alpha-cyclodextrin from MO seeds	SH-SY5Y human neuroblastoma cells	Inhibition of PI3K/Akt/mTOR pathway, (NF- κ B) p65 activation and phosphorylation; downregulated MAPK pathway; apoptotic death triggered by MAC pathway (based on expression level of cleaved-caspase 3, Bax/Bcl-2 balance, p53 and p21)	Anti-proliferative efficacy, apoptosis induction
Antonini et al., 2018 [56]	Seeds (moringin (MOR), a glycosyl-isothiocyanate obtained by myrosinase-catalyzed hydrolysis of the precursor 4-(α -l-rhamnosyloxy)-benzyl glucosinolate or glucomoringin)	Hep3B cancer cells	AVN 2f-mediated caspase-8 activation (Extrinsic apoptosis), MOR-mediated increased level of intracellular ROS, caspases 2 and 9 activation, and downregulation of pro-survival gene BIRC5	Anti-proliferative and pro-apoptotic effects
Asaduzzaman et al., 2018 [57]	Seed (Purified lectin)	Mouse model injected by Ehrlich ascites carcinoma (EAC) cells	Inhibited cell cycle at G2/M phase and caspase-3, suppressed expression of Bcl-2 and NF- κ B genes, the activation of Bak	Anti-proliferative effect, apoptosis induction

Continued Table 1.

Reference	Formulation	Cell line/Animal model	Pathway	Outcome
Jaafaru et al., 2018 [58]	Pod (GMG-ITC rich soluble extract)	Sprague-Dawley (SD) rats, murine fibroblast (3T3), and PC-3 human prostate adenocarcinoma cells	Induced apoptosis	Anti-proliferative effect
Hagoel et al., 2019 [59]	Leaf (aqueous extract)	PANC-1 human pancreatic epithelioid carcinoma cell line, Ectopic (subcutaneous) tumor model in immune deficient athymic CD-1 nude mice	Decreased expression of pro-apoptotic protein Bcl-2 in combination with downregulation of PARP-1 and NF- κ B-related proteins I κ B- α , p65-subunit, and COX-2	Anti-proliferative activity, anti-angiogenic effect, and enhancing the radiotherapeutic efficacy against tumor radioresistance
Paul et al., 2019 [60]	Leaf (bis (Isothiocyanatomethyl) benzene)	HeLa human cervical cancer cells, MCF-7 human breast adenocarcinoma cells, and MDA-MB-231 cells	Caspase 3 apoptotic pathway	Antitumor activity through establishing cancer cell growth and inhibiting carcinogenesis
Kumar et al., 2019 [61]	Leaf (n-hexane extract)	A549 non-small-cell lung cancer cells	Cytotoxicity	Anti-oxidant and antitumor activity
Potestà et al., 2019 [62]	Seed and Leaf (boiled and frozen aqueous extracts)	Human Jurkat E6-1 lymphoid and THP1 monocytoid cell lines; peripheral blood mononuclear cells (PBMCs) from healthy donors	Anti-proliferative and pro-apoptotic effects	Aqueous extracts differentially regulated proliferation and apoptosis in healthy cells and cancer cells, and they can be used as adjuvant in cancer therapy
Tiloke et al., 2020 [63]	Leaf (crude aqueous extract)	Human liver hepatocellular carcinoma (HepG2) cells; PBMCs; HEK293 (human embryonic kidney)	Apoptosis was assessed by caspase-9, -3/7 activities and ATP levels. Cell cycle, γ H2AX, and cleaved PARP-1 were determined. Protein expression of c-myc, Bax, p-Bcl2, Smac/DIABLO, Hsp70, SRp30a and cleaved PARP-1 was assessed	Cell-cycle arrest and apoptosis in cancerous HepG2 cells
Khan et al., 2020 [64]	Leaf (methanolic extract)	PC-3 prostate cancer cells	Inhibiting Hedgehog signaling, cell cycle arrest in G0/G1, and significant alteration in mRNA expression of apoptosis-related and Hedgehog signaling pathway genes	Inhibitory effects against cancer cells
Luetragoon et al., 2020 [19]	Leaf (ethyl acetate extracts and their fractions, 3-hydroxy- β -ionone)	Squamous cell carcinoma SCC15 cell line	Cell viability on SCC15. Cell cycle and apoptosis markers were evaluated by immunoblotting	Anti-Cancer Effect of 3-Hydroxy- β -Ionone; antiproliferative
Dilworth et al., 2020 [65]	Leaf extract	Human promyelocytic leukemia cells (HL60 cells) subjected to oxidative stress	Viability assays; Antioxidant indexes	Protection from oxidative stress within the first 24 h, as well as increasing cell viability at certain concentrations
Xie et al., 2020 [66]	Leaf (alkaloids)	PC3 human prostate cancer cell line	Suppressing COX-2-mediated Wnt/ β -catenin signaling pathway	Anti-migration, anti-proliferation
Aldakheel et al., 2020 [67]	Seed (ethanol extract)	Human colorectal carcinoma cells (HCT-116) cells	DNA nuclear morphology	Inhibitory action
Phannasil et al., 2020 [68]	Pod (dietary boiled extract)	Azoxymethane and dextran sodium sulfate-induced mouse colon carcinogenesis	Histological assay and colon proteomic assay; network analysis	Both preventive and therapeutic antitumor effects
Patriota et al., 2020 [69]	Flower (<i>Moringa oleifera</i> (drumstick tree) flower trypsin inhibitor (MoFTI) derived from protein extract)	Sarcoma 180-bearing mice	Tumor weight and vasogenesis	Reduced tumor weight and a reduced number of secondary vessels and lower gauge of the primary vessels
Wisitpongpun et al., 2020 [70]	Leaf (n-hexane, ethyl acetate and 95% ethanol extract and their fractionation)	MDA-MB-231 Cell Line	Apoptosis and cell cycle analysis	A fraction derived crude ethyl acetate extract reduced cell viability and clonogenic growth and increased cells apoptosis.
Do et al., 2020 [17]	Leaf (aqueous extract)	A375 cells and A2058 melanoma cells	Apoptosis which was expressed via chromatin condensation and PS externalization; decreased mitochondrial membrane potential; increased Bax/Bcl-2 ratio, activated Caspase-3/7, Caspase-9, PARP and AIF translocation, leading to apoptotic cell death	Anti-proliferation, apoptosis induction

Continued Table 1.

Reference	Formulation	Cell line/Animal model	Pathway	Outcome
Lim et al., 2020 [71]	Leaf and seed (the mixture of MO leaves and seed residue (MOLSr) extract)	MCF-7 breast cancer cells, PMECs primary mammary epithelial cells, non-malignant Chang's liver cells, HepG2 human hepatocellular carcinoma, HeLa cervical adenocarcinoma cells, and MCF-7 human breast adenocarcinoma cells	Significantly reduced expression of calcineurin (CaN) and vascular endothelial cell growth factor (VEGF) proteins as well as secreted frizzled-related protein 1 (SFRP1) and solute carrier family 39 member 6 (SLC39A6) genes.	Chemopreventive activity
Pandey and Khan, 2020 [72]	Leaf (methanolic extract)	HeLa Human cervical cancer cells	Down-regulated Jab1 expression, significantly increased p27 expression, enhanced ROS production and caspase-3 activation	Cell cycle arrest, targeting signalosome (Jab1) in cancer cells
Barhoi, et al 2021 [73]	The aqueous extract of MO (AEMO)	EAC (Ehrlich acites carcinoma) solid tumor-bearing mice and HEP-2 (human laryngeal carcinoma cells)	Apoptosis induction through changing mitochondrial membrane potential	The inhibition of tumor progression
Fuel et al., 2021 [74]	Seeds (ethanolic extract)	Colorectal cancer (CRC) cell lines T84, HCT-15, SW480 and HT-29, as well as with cancer stem cells (CSCs)	Overexpression of caspases 9, 8 and 3 and increased production of reactive oxygen species	Extracts presented the lowest IC50 in T-84 and HCT-15 (resistant) cells, highest level of inhibition of proliferation in multicellular tumor spheroids of HCT-15 cells. Synergistic activity with 5-Fu (5-Fluorouracil), cell death by autophagy
Siddiqui et al., 2021 [75]	Fruit (ethanolic extract)	HepG2 human hepatocellular carcinoma cells	ROS-mediated apoptosis induction and activation of caspase-3	Anti-proliferative, apoptosis induction
Zunica et al., 2021 [76]	Seed (ethanol extract dried concentrate)	Immunocompromised female mice with diet-induced obesity bearing MDA-MB-231-derived xenograft tumors	Cytotoxicity assay	Anti-angiogenesis
Cuellar-Núñez et al., 2021 [77]	Leaf (lyophilized powder supplement)	Azoxymethane-induced colitis-associated colon carcinoma model in CD-1 mice	Down-regulated expression of pro-inflammatory mediators including IL-2, IL-6, TNF, IL-1 β , and INF- γ ; as well as reduced pro-inflammatory cytokines (MCP-1, IL-6, TNF- α) in serum	Chemo-preventive, anti-inflammatory
Do et al., 2021 [18]	Leaf (methanolic extract, n-hexane, chloroform, ethyl acetate, and water-soluble fractions)	A375 human melanoma cells, A2058 human melanoma cells, and WS1 normal human skin fibroblasts	WST-1 assay, The caspase-dependent and caspase-independent pathways involved in apoptosis	Anti-proliferation, apoptosis induction
Xie et al., 2021 [78]	Leaf (extraction of alkaloids from leaf powder via using various ethanol and ethyl acetate solvents)	A549, HCT116, A375, MDA-MB-231, Hep-G2, NCI-H1975, NCI-H1781, NCI-H441, and GES-1 cells A549 lung cancer cells, GES-1 cells (human gastric mucosal epithelial cells).	The proliferation, apoptosis, cell cycle, and migration of cells; expression of caspase-3 and caspase-9, cyclin D1 and cyclin E	Strongest growth inhibitory effect against A549 cells but had low toxicity to GES-1 cells; induced cell cycle arrest; anti-migration, anti-proliferation, and apoptosis induction
Mohd Fisall et al., 2021 [79]	Leaf (methanolic extract)	MCF-7 Human breast adenocarcinoma cells	Increased expression of pro-apoptotic proteins including Bax, caspase 8 and p53; cytotoxicity assay	Anti-proliferation, apoptosis induction
Caicedo-Lopez et al., 2021 [80]	Leaf (fermented non-digestible fraction)	HT-29 colorectal cancer cells	Induced apoptosis, autophagy, and necrosis	Anti-proliferation
Lu et al., 2021 [81]	Seed (moringin isolated and purified from defatted moringa seed powders and its degraded products were prepared)	Three cancer cell lines (MCF-7, HepG2 and HCT-116)	Cytotoxicity	Moringin showed good cytotoxicity to cancer cells, however its degraded products showed very weak or no activity
Bhadresha et al., 2021 [82]	Leaf (digested and non-digested extracts)	Human prostate bone metastasis cancer cell line PC3	The blockage of cell cycle in S phase; Cell proliferation assay; apoptosis assay via acridine orange and propidium iodide double staining	Cytotoxicity, cell cycle arrest, and apoptosis induction

refine drinking water [32]. The aqueous seed extract and cMol were potentially cytotoxic to human peripheral blood mononuclear cells, whereas WSMoL and diluted seed extract did not show any cytotoxic activity [32]. In addition, the MO aqueous seed extract and the lectins cMol and WSMoL were weakly/moderately cytotoxic to HT-29 human colorectal adenocarcinoma and HEP-2 human epithelial type 2 cancer cell lines [32]. Furthermore,

the aqueous seed extract, cMol and WSMoL and diluted seed extract exhibited anti-inflammatory activity [32].

Another study has been focused on RMCCA1 and KKU100 human hilar cholangiocarcinoma cell lines treated with MO extracts which examined cell viability and apoptosis [37], showed that MO extracts inhibited cellular proliferation and induced cell apoptosis. Additionally, the leaf and seed extracts of MO incited

cytotoxicity through a marked decrease in mitochondrial membrane potential and an increase in ROS production in cholangiocarcinoma cells.

The antiproliferative assay conducted on MO leaf demonstrated that dichloromethane extract have a higher antiproliferative activity compared to methanol extract on HepG2 and colorectal adenocarcinoma (Caco-2) cell lines [34]. In addition, the assessment of *in vitro* chemopreventive activity against cancer cells was implemented through quinone reductase (QR) induction assay as a useful strategy on hepatoma (Hepa-1c1c7) which revealed that the dichloromethane extract had the capacity to induce QR activity significantly, whereas the methanol extract appeared to have no inductive effect [34]. In a nutshell, this study provides evidence that MO leaves possess anti-oxidantive, cytotoxic and chemopreventive properties [34].

The *in vitro* antiproliferative potential of MO extracts against HCT-16 colorectal carcinoma cells and Colo-205 Dukes' type D, colorectal adenocarcinoma cells; has been confirmed using SRB and MTT tetrazolium reduction assays [40]. The antitumor activity of MO leaf extracts against HepG2 cells has been reported [41]. In a labor-intensive work, n-hexane, chloroform, ethyl acetate, methanol extracts of the MO leaves and 15 fractions (F1 to F15) of ethyl acetate extract were evaluated *in vitro* and *in vivo* for their antitumor activity using Hep-2 cell lines and Dalton's lymphoma ascites model in mice, respectively [42]. Authors stated the potential cytotoxic impact of F1 fraction on Hep-2 cell lines, a significantly reduced body weight, and increased mean survival time in the animal model of Dalton's lymphoma ascites.

The different cell lines including HepG2, HeLa, and Caco-2 were treated with essential oil derived from MO seeds with a range of concentrations from 0.15 to 1 mg/mL for 24h; additionally, the cytotoxicity was assessed using

MTT assay. Findings showed a significant reduction in cell viability in response to the ascending dose of essential oil in all treated cell lines [38].

The viability of Caco-2 and HepG2 cells were reduced by treating with aqueous leaf extract of MO [49]. In a pioneering study, the cytotoxicity caused by the major aromatic multi-glycosylated glucosinolates of MO leaf extracts, namely 4- α -rhamnopyranosyloxy-benzyl glucosinolate (Rhamno-Benzyl-GS) and acetyl-4- α -rhamnopyranosyloxy-benzyl glucosinolate Isomer III (Ac-Isomer-GS III), to HepG2 cells and two Salmonella typhimurium strains (Ames test), was confirmed. As per the findings of this study, no genotoxicity caused by glucosinolates was reported through Ames test; nevertheless, stimulating effects produced by these glucosinolates have been corroborated both at the transcriptional and translational levels of antioxidative systems such as NRF2 (nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2) pathway [48]. In addition, the MO crude aqueous leaf extract showed antiproliferative effects on esophageal cancer cell line (SNO) through the augmentation of DNA fragmentation, lipid peroxidation, and the induction of apoptosis [46].

Quercetin-3-O-glucoside and 4-(β -D-glucopyranosyl-1 \rightarrow 4- α -L-rhamnopyranosyloxy)-benzyl isothiocyanate, isolated from the leaves and seeds of the MO from Kenya, showed antioxidative and cytotoxic activities against HepG2 and Caco-2 cell lines [44]. In this respect, one study reported that MO leaves which contain dietary fiber and phenolic compounds exerted a suppressive effect on an *in vivo* model of AOM/DSS-induced colorectal carcinogenesis [50].

The antiproliferative activity against RMCCA1 cholangiocarcinoma cells promoted by MO seeds extract has been confirmed with the cell proliferation assay [53]. The activation of protein kinase B (Akt) has been demonstrated in RMCCA1 cells pre-treated with the

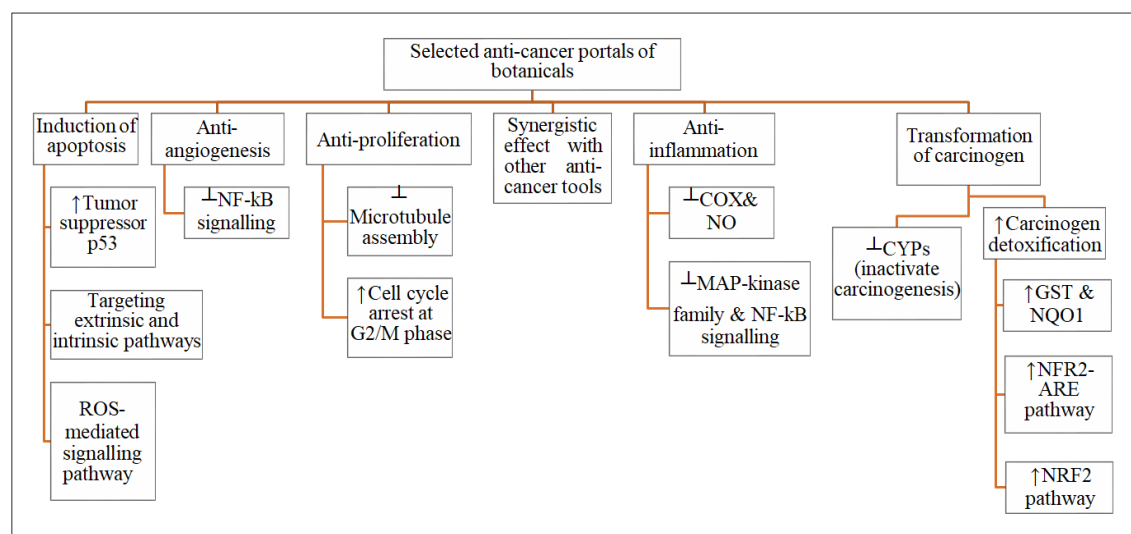


Figure 1. The Major Anti-cancer Portals of Botanicals. \downarrow , Inhibition; \uparrow , Activation; CYPs, Cytochrome P Enzymes; GST, Glutathione-S-Transferase; NQO1, Quinone Oxidoreductase 1; NRF2, Nuclear Transcription Factor Erythroid 2p45 (NF-E2)-related Factor 2 Signaling Pathway; ARE, Antioxidant Responsive Elements; COX, Cyclooxygenase; NO, Nitrogen Oxidase; MAP, Microtubule-associated Protein; NF- κ B, Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B cells; ROS, Reactive Oxygen Species.



Figure 2. *Moringa oleifera* Lam. Moringaceae cultivated in Laybid, Shahin Shahr, Isfahan, IRAN

ethanolic extracts of MO seed [53]. *Moringa oleifera* leaf ethanol extract showed hepato-protective effects upon diethyl nitrosamine (DEN)-induced hepatocellular carcinoma may have been mediated by its antioxidative and apoptosis-promoting activities [51].

Reportedly, a fermented MO extract exerted an ameliorative effect upon glucose intolerance and mitigated the hepatic adiposity in the high-fat diet-induced non-alcoholic fatty liver disease, by means of decreasing endoplasmic reticulum stress, oxidative stress, and inflammation in C57BL/6 mice [83].

In another study, the *in vitro* antiproliferative effect and the mechanism of action by which successive fractions from the methanol extract of MO leaves exerts, have been investigated on HCT116 human colon carcinoma cell line [52]. Results demonstrated the presence of astragaloside and isoquercetin in some fractions and significantly suppressed cell proliferation through the downregulation of ERK1/2 phosphorylation caused by them.

In a noteworthy study, the acute toxic and apoptosis-promoting effects of MO soluble extracts (GMG-ITC-RSE) replete with glucomoringin-isothiocyanate were investigated both *in vivo* and *in vitro*, respectively [84]. In this continuum, GMG-ITC-RSE did not appear to prompt adverse effects to rats even at high doses (2000 mg/kg body weight). Non-toxic GMG-ITC-RSE, triggered significant morphological aberrations indicative of apoptosis which confirmed its antiproliferative effect on PC-3 human prostate adenocarcinoma cell line.

Moringin (MOR), a glycosyl-isothiocyanate obtained through the myrosinase-catalyzed hydrolysis of the precursor 4-(α -l-rhamnosyloxy)-benzyl glucosinolate (glucomoringin), found predominantly in the seeds of MO, showed antiproliferative and pro-apoptotic effects upon Hep3B cancer cells resistant to anti-neoplastic agents. In this regard, the intrinsic apoptotic pathway was triggered by MOR-induced intracellular increase in ROS, the MOR-mediated activation of caspases 2 and 9, and the MOR-mediated downregulation of the pro-survival gene BIRC5 [56].

Another study has been focused upon the effect of MO in combination with radiation both on survival and metastasibility of pancreatic cancer cells, and on tumor growth [59]. The authors concluded that the combination of MO and radiation significantly inhibited PANC-1

cell survival in a dose-dependent manner, reduced metastasibility of these cells, and inhibited the growth of subcutaneous tumors engendered by PANC-1 cells in nude mice [59].

Tiloke and colleagues (2019) reported MO crude aqueous extracts displayed minimal cytotoxicity in peripheral blood mononuclear cell and human embryonic kidney (Hek293) cells while it led to increased lipid peroxidation, DNA damage and γ H2AX levels with a decrease in G1, S and G2-M phase for 24 h in human liver HepG2 cells. Finally, they concluded that MO extracts induced cell-cycle arrest and apoptosis in cancerous HepG2 cells [63].

Another impressive study has put its concentration upon the potential antitumor effect of the aqueous extract of MO in mice bearing both EAC (Ehrlich ascites carcinoma)-induced solid tumor and tumor composed of HEp-2 human laryngeal carcinoma cells, and antitumor activity was reported. A vast range of phytochemicals including quinic acid, octadecanoic acid, hexadecanoic acid (palmitic acid), α -tocopherol (vitamin E), and γ -sitosterol, as the predominant bioactive components of the aqueous extract of MO, was reported [73].

Aldakheel and colleagues (2020) showed inhibitory action of seed ethanol extract of MO against human colorectal carcinoma cells (HCT-116) cells beside its biosafety for HEK-293 cells (non-cancerous cells) [67].

Phannasil et al. (2020) dogged deeper to decipher the antitumor activity of boiled MO pod-supplemented diets on azoxymethane/dextran sodium sulfate induced mouse colon carcinogenesis [68]. They reported the protein expression profiles responsible for the suppressive effect of this supplementation including 125 proteins that were differentially expressed in mouse colon tissues potentially involved in regulating apoptotic and inflammatory signaling networks in colorectal cancer prevention and therapy.

Caicedo-Lopez and colleagues (2021) reported antitumor activities of the impact of the colonic metabolites obtained from the fermented non-digestible fraction MO leaves on cell death mechanisms from human colorectal adenocarcinoma cell line (HT-29) cells [80]. In this scenario, this novel fermented formula exerted antiproliferative effect against HT-29 colorectal cancer cells via induction of increased H_2O_2 production, apoptosis, autophagy, and necrosis.

Fuel et al. (2021) analyzed chromatographically ethanolic extracts of the seeds of MO and declared that its extracts had excellent capacity to enhance activities of antioxidative enzymes such as glutathione S-transferase and QR [74]. they showed antitumor activity of this MO extracts against a bunch of colorectal cell lines and more surprisingly the showed its antitumor activity against cancer stem cells (CSCs) which are self-renewable cell types that are identified in most types of liquid and solid cancers and contributed to tumor onset, expansion, resistance, recurrence, and metastasis after therapy. This seminal study will open a research avenue to make MO-derived formulations as a candidate for clinical studies for human or veterinary mono- or poly-oncotherapy since

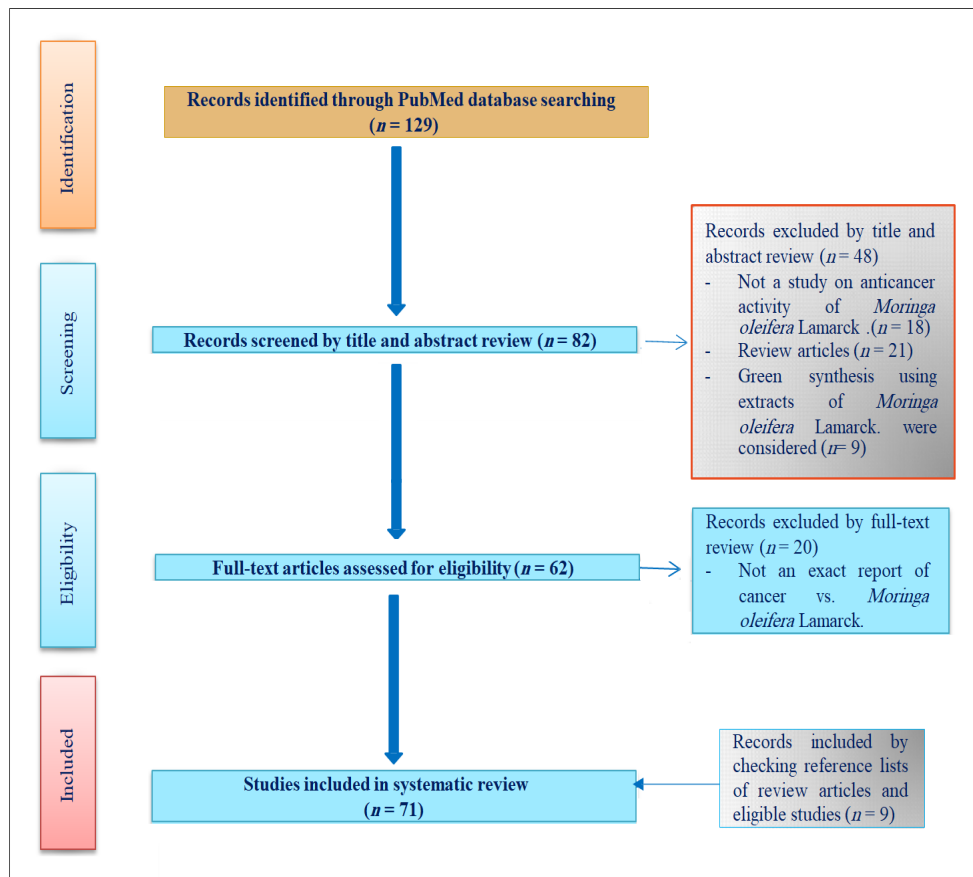


Figure 3. Flow Diagram of the Literature Search on the Antitumor Effect of *Moringa oleifera* Lam. in Preclinical Studies

this extract showed synergism with 5 fluorouracil.

Siddiqui et al. (2021) reported the antiproliferative activity of MO fruit extract on human liver cancer HepG2 cells and computational validation of cell death through ROS-mediated apoptosis and activation of caspase-3 enzyme [75]. They introduced benzyl glucosinolate as the best binders of caspase-3 enzyme while we cannot discriminate among activators or inhibitors in molecular docking and further experimental studies will (dis)prove this impressive finding. Cuellar-Núñez et al. (2021) experimentally showed that intake of a supplement contained 10% MO leaf powder induced differential expression of 65 genes in colon tissue such as IL-2, IL-6, TNF, IL-1 β , and INF- γ [77]. In this context, MO downregulated proinflammatory mediators showing chemopreventive properties against inflammatory response and colon carcinogenesis.

Respiratory cancers

A weak/moderate cytotoxicity to the NCI-H292, human mucoepidermoid pulmonary carcinoma cancer cell lines, was reported to be induced by both aqueous seed extract (ASE) of MO and the lectins cMol and WSMoL [32]. Both leukocyte migration and the number of leukocytes present in the lungs were diminished by ASE [32]. The cytotoxicity of ASE and cMol to immune cells may be the case and this supports the hypothesis of extract having immunosuppressive activity [32]. The antiproliferative effect of MO leaf extract in A549 lung

cancer cells has been confirmed to be associated with the enhancement of oxidative stress, DNA fragmentation and apoptosis [31]. The MO aqueous leaf extract exerts its cytotoxic effect on A549 adenocarcinomic human alveolar basal epithelial cells by means of affecting the mitochondrial viability and inducing apoptosis through a ROS-dependent manner [49]. The MO water extract greatly promoted apoptosis, inhibited tumor cell growth, and reduced the level of internal ROS in human lung cancer cells and other types of cancer cells including A549 adenocarcinomic human alveolar basal epithelial

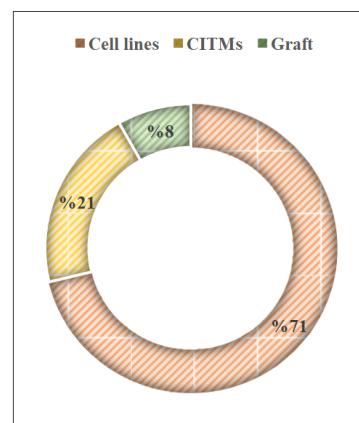


Figure 4. The Typology of Studies focused on Anti-cancer Effects of *Moringa oleifera* Lam. CITMs: Chemically Induced Tumor Model

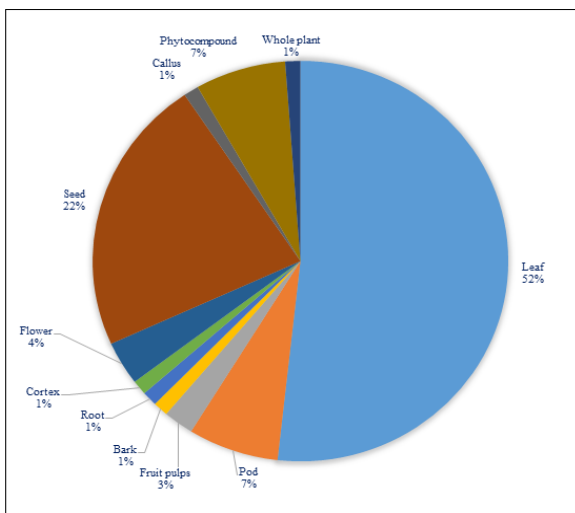


Figure 5. The Classification of Selected Studies Based on the Part Used for Evaluation of Anti-cancer Potential of *Moringa oleifera* Lam.

cells, H23 human adenocarcinoma (non-small cell lung carcinoma), and H358 human bronchoalveolar carcinoma (non-small cell lung carcinoma) cell lines [15].

The *in vitro* antiproliferative activity of MO extracts against a diverse range of cancer cell lines such as A549 adenocarcinomic human alveolar basal epithelial cells, has been confirmed using sulphorhodamine-B (SRB) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assays [40].

In another ground-breaking study, two phytocompounds derived from MO leaf extracts, namely 4- α -rhamnopyranosyloxy-benzyl glucosinolate (Rhamno-Benzyl-GS) and acetyl-4- α -rhamnopyranosyloxy-benzyl glucosinolate Isomer III (Ac-Isomer-GS III), were reported to have cytotoxic effects upon V79-MZ Chinese hamster lung cells [48].

In the other study, antioxidative activity and moderate antitumor effect of various MO extracts have been reported in A549 adenocarcinomic human alveolar basal epithelial cells [61].

Xie et al. (2021) reported antitumor activity of an alkaloid extract of leaves of MO on a bunch of cancer cell lines and they concluded that this extract exerted the strongest growth inhibitory effect through inducing apoptosis and increasing the expression of the apoptosis-related proteins caspase-3 and caspase-9 against A549 cells but had low toxicity to GES-1 cells (human gastric mucosal epithelial cells), therefore this extract has potential for therapy and prophylaxis of lung cancer [66].

Neurological cancers

Neuro-protective effect of glucomoringin-isothiocyanate (GMG-ITC) also known as moringin suggests that GMG-ITC has the potential to protect the neuronal integrity in response to cytotoxicity associated with increased oxidative stress which is recognized as one of the hallmarks of neurodegenerative diseases [58].

Polar extracts prepared out of dried and milled leaves of MO grown in Jamaica showed significantly

higher antioxidative activity through the assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) than those of other phytocompounds [85].

The *in vivo* and *in vitro* antiproliferative activity of an MO seed lectin (MOSL) was investigated to examine whether it exerts any activity against the proliferation of Ehrlich ascites carcinoma (EAC) cells, and the results showed dramatic inhibitory effects [57]. In particular, isothiocyanates (ITCs), which exert antitumor effects; and a newly prepared MO-derived 4-(α -L-rhamnopyranosyloxy) benzyl ITC (moringin) combined with alpha-cyclodextrin (moringin + α -CD; MAC) decreased the growth of SH-SY5Y human neuroblastoma cells in a concentration-dependent manner specifically through inhibiting the PI3K/Akt/mTOR pathway, whose aberrant activation was found to be associated with numerous types of tumors [55].

The *in vitro* antitumor effect caused by glycosylated isothiocyanate moringin (4-(α -l-rhamnopyranosyloxy) benzyl isothiocyanate), produced through the quantitative myrosinase-induced hydrolysis of glucomoringin (GMG) under a neutral pH value, upon human astrocytoma cells has been reported [45].

Genitourinary cancers

In another study, the antitumor effects of Saudi Arabian MO leaves, bark and seed extracts have been investigated against MDA-MB-231, a “basal” type and triple negative (ER, PR and HER2 negative) breast cancer cell line [39]. The authors found that in spite of neutral effect of seed extracts, the extracts of leaves and bark exhibited remarkable antitumor properties through decreasing cell survival, dramatic decrease (about 70–90%) in colony formation, diminished cell motility, increased apoptosis, and arrested progression of cell cycle at the G2/M phase. Coupled with aforementioned descriptive findings, the authors proposed that the anti-neoplastic effects of MO extracts are associated with a vast spectrum of compounds chemically detected in MO extracts and reportedly showing antitumor activity including eugenol, isopropyl isothiocyanate, D-allose, and hexadecanoic acid ethyl ester [39].

The leaf extract of MO showed both dose- and time-dependent antiproliferative effect in MCF-7 human breast adenocarcinoma cell line and MDA-MB-231; a highly aggressive, invasive and poorly differentiated triple-negative breast cancer; comparable to Cis-Platinum [33]. The MO soluble cold aqueous extract significantly induced apoptosis, inhibited tumor cell growth, and lowered the level of internal ROS in MCF-7 human breast adenocarcinoma cell line [15]. The *in vitro* antiproliferative effect of MO extracts upon a wide spectrum of cancer cell lines including PC-3 prostate cancer cells, T47D human ductal carcinoma cells, and MCF-7 human breast adenocarcinoma cells, have been confirmed using SRB and MTT tetrazolium reduction assays [40]. The chemopreventive effect incited by the hydro-ethanolic extract of MO pods on DMBA-induced renal carcinogenicity may be mediated through its antioxidative properties [29].

Adopting a mechanistic perspective, the antiproliferative and apoptotic effect of benzyl isothiocyanate (BITC) upon ovarian cancer (OC) have been investigated. In line with this, BITC inhibited the proliferation of OC cells and made them undergo apoptosis as well [86]. Reportedly, the dichloromethane extract of MO leaf showed higher antiproliferative effect on MCF-7 (human breast adenocarcinoma cell line) of MO leaf was assessed and it was revealed that dichloromethane extract has higher activity in comparison with that of methanol extract on MCF-7 breast adenocarcinoma cell lines [34].

The MCF-7 human breast adenocarcinoma was treated with the essential oil extracted from MO seeds at a range of concentrations from 0.15 to 1 mg/mL for 24h, and the cytotoxicity was assessed through the MTT assay. Findings revealed that cell viability was significantly reduced in response to the ascending dose of essential oil in the treated cell line [38].

The aqueous leaf extract of MO decreased the viability of HEK293 human embryonic kidney 293 cells as well [49]. The aqueous extracts of MO leaf and flower produced different effects depending upon which cells they are being treated with [43]. The aqueous leaf extract brought about a moderate increment in the proliferation of mesenchymal stem cells (MSCs), angiogenesis, and a substantially enhanced hepato-protectivity, whereas it showed prominent cytotoxicity to cancer cell line (MCF-7). The aqueous extract of MO flower showed to a stimulatory effect on cellular proliferation; however, the same effect on cancer cell lines was not exerted. In addition, the leaf extract, had a prominent antitumor and hepato-protective effects [43].

The hydro-alcoholic extract of MO seeds and its fractions with four solvents (n-hexane, dichloromethane, chloroform, and n-butanol), significantly inhibited the cellular growth which has been tested on MCF 10A (human fibrocystic disease) cell line. Crude water extract, n-hexane and dichloromethane fractions of the

seeds inhibited the proliferation of MCF-7 cells with half-maximal inhibitory concentration (IC₅₀) of 280 µg/ml, 130 µg/ml and 26 µg/ml respectively; however, amongst the 3 samples, n-hexane fraction had the minimal cytotoxic effect on MCF 10A (IC₅₀ > 400µg/ml) [54].

The bis (isothiocyanatomethyl) benzene, an anti-neoplastic phytochemical, has been purified from MO leaf extract which suppresses cancer cell growth and imposes inhibitory effect upon carcinogenesis within HeLa, MCF-7, and MDA-MB-231 cell lines by means of caspase 3 apoptotic pathway. Although not being quite similar, it bears much resemblance to drug Cis-Platinum, an anti-neoplastic agent available at the market [60].

Khan and colleagues (2020) proposed the inhibitory potential of MO methanolic extract of leaves against PC-3 prostate cancer cells via inhibiting hedgehog signaling pathway. They also observed a dose-dependent G₀/G₁ cell cycle arrest and a significant alteration in mRNA expression of genes related to apoptosis and hedgehog signaling pathway following the treatment with MO extract [64].

In a seminal work, Wisitpongpan and colleagues (2020) performed bioassay-guided fractionation and documentation of phytochemicals derived from MO leaf have been assessed against MDA-MB-231 breast cancer cells. The authors reported that the best anticancer activities were related to a fraction derived from crude ethyl acetate extract [70]. This fraction considerably reduced cell viability and clonogenic growth and increased apoptosis in aforementioned cell line and it possessed a plethora of phytochemicals among them 7-octenoic acid, oleamide, and 1-phenyl-2-pentanol showed anticancer activities by inducing cell cycle arrest and triggering apoptosis through suppressed Bcl-2 expression which subsequently promoted activation of caspase 3, an indicator for the apoptosis pathway.

Pandey and Khan (2020) reported the inhibitory role of methanolic extract of MO leaves against Jab1 signalosome in cervical cancer by analyzing several *in vitro* battery tests which reflected decreased cell growth throughout inducing apoptosis in a dose and time dependent manner [72].

Xie et al. (2020) evaluated mechanistically the inhibitory effect of MO alkaloids (MOA) on proliferation and migration of PC3 human prostate cancer cells *in vitro* and *in vivo* [66]. The results showed that MOA inhibited proliferation of PC3 cells and induced apoptosis and cell cycle arrest beside suppression of PC3 cell migration, inhibition of the expression of matrix metalloproteinases (MMP)-9, downregulation of the expression of cyclooxygenase 2 (COX-2), β-catenin, phosphorylated glycogen synthase 3β, and vascular endothelial growth factor, and suppression of production of prostaglandin E₂ (PGE₂) and reversion of PGE₂-induced PC3 cell proliferation and migration. Furthermore, *in vivo* experiments showed that MOA significantly inhibited growth of xenograft tumors in mice, and significantly reduced the protein expression levels of COX-2 and β-catenin in tumor tissues.

Lim et al. (2020) investigated chemopreventive values of the combined mixture of MO leaves and seed residues

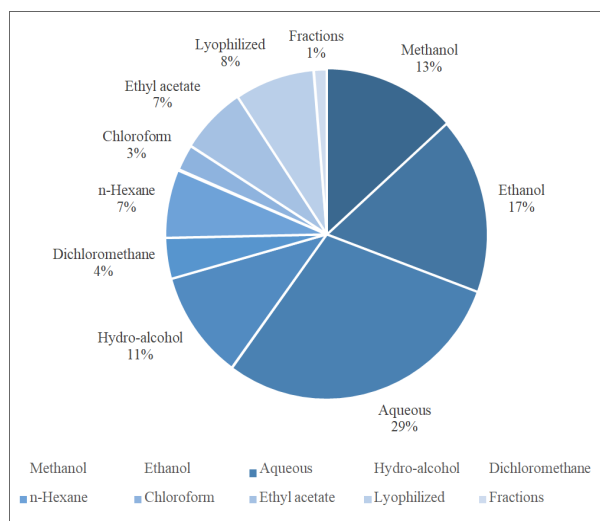


Figure 6. The Classification of Selected Studies Based on the Solvent Used for Evaluation of Anti-cancer Potential of *Moringa oleifera* Lam.

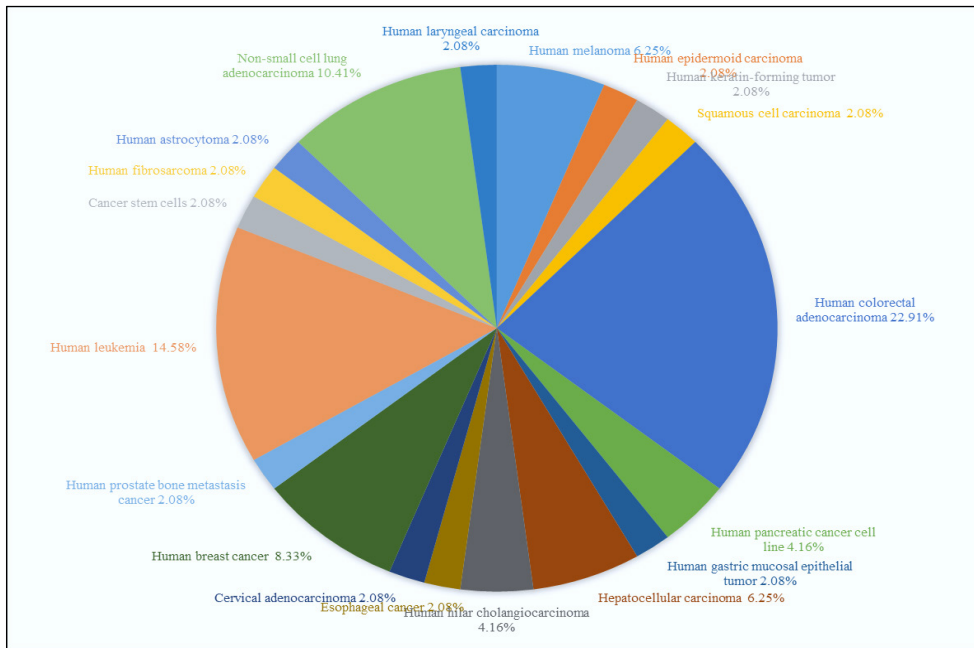


Figure 7. The Classification of Selected Studies Based on the Cancer Phenotype Used for Evaluation of Anti-cancer Potential of *Moringa oleifera* Lam.

on the primary mammary epithelial cells (PMECs), non-malignant Chang’s liver cells and various human cancer cell lines (including breast, cervical, colon and liver cancer cell lines) [71]. They also tried this mixture in xenograft mice injected with MDA-MB-231 cells for *in vivo*. This mixture showed chemopreventive or cytotoxic effects on in MCF-7 breast cancer cells and PMECs with alteration of the levels of candidate genes involving in tumorigenesis.

Zunica et al. (2021) focused on the antitumor effects of dietary MO seed supplementation in immunocompromised female mice with diet-induced obesity bearing MDA-MB-231-derived xenograft tumors [76]. More surprisingly,

moringa supplementation could not disturb tumor progression and authors concluded that consumption of moringa seed extracts while receiving chemotherapy for breast cancer treatment should be cautiously assessed. The striking feature of this study was using triple negative breast cancer which is an aggressive and highly metastatic breast cancer subtype with limited treatment options.

Mohd Fisall et al. (2021) prepared various leaf extracts and fractions of MO and assessed their cytotoxic and apoptotic effects on breast cancer (MCF7) cells [79]. They finally reported that the dichloromethane extract was selectively cytotoxic to MCF7 cells (5 µg/mL) without significantly inhibiting the non-cancerous breast (MCF

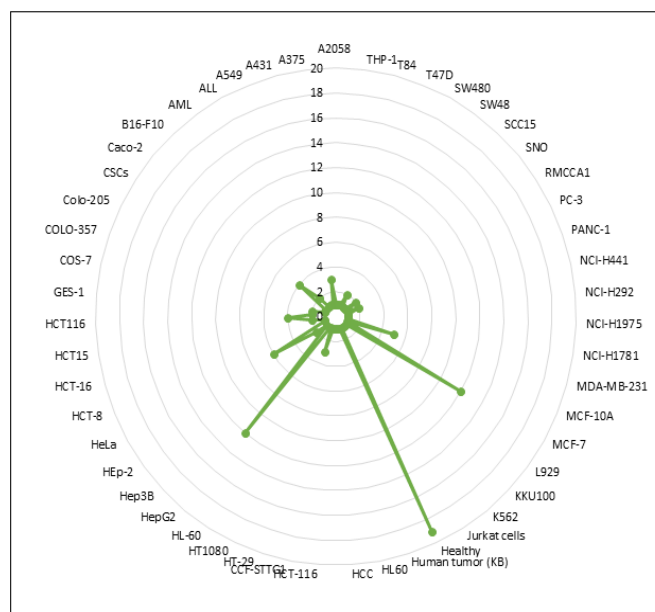


Figure 8. The Classification of Selected Studies Based on the Cell Lines Used for Evaluation of Anti-cancer Potential of *Moringa oleifera* Lam.

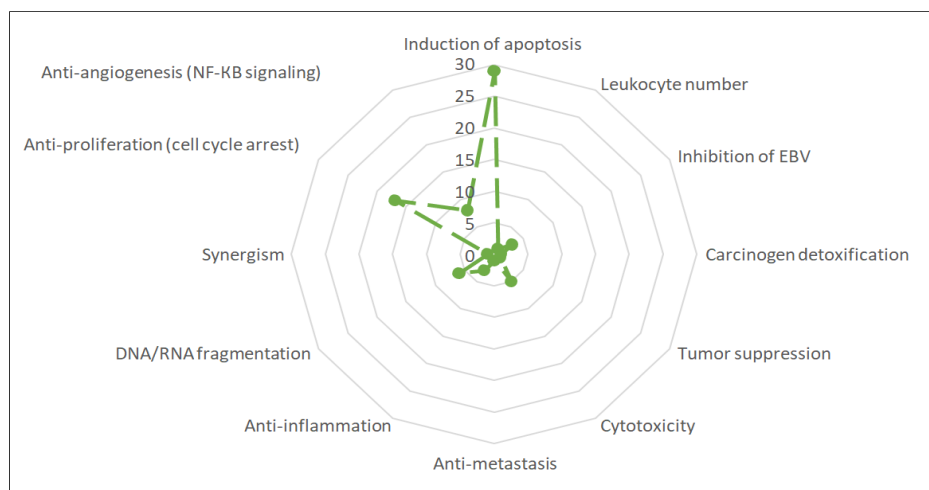


Figure 9. The Major Anti-cancer Portals of *Moringa oleifera* Lam.

10A) cells.

Bhadresha et al. (2021) investigated digestion effects on the bio-efficacy of leaves of MO on a PC3 cell line and finally they concluded that although *in vitro* digestion will decrease antioxidative potency of digesta but potentiates its antitumor effects against prostate cancer bone metastasis. This study will open a research aperture to the pharmacology of oral consumption of MO leaves as functional table vegetable in daily food menu.

Hematological cancers

Cold, hot, and ethanol extracts prepared from MO leaves could kill the majority (70 - 86%) of the cells among primary cells harvested from the clinical samples belonging to patients suffering from acute lymphoblastic leukemia and acute myeloid leukemia [20]. In this regard, hot water and ethanol extracts were more effective against abovementioned cancer cells.

The clastogenic and anti-clastogenic effects of freeze-dried boiled MO pod (bMO) were assessed in the presence of direct-acting clastogens (mitomycin C, MMC) and an indirect-acting agent (DMBA) using the *in vivo* erythrocyte micronucleus assay in mice [27]. Authors reported that freeze-dried bMO not only showed no clastogenicity but also produced an anti-clastogenic effect, particularly on the direct-acting clastogen in mouse [27]. The chemopreventive and anti-leukemic effects induced by the ethanolic extracts of MO leaves on benzene-induced leukemia, have been reported in rats [36]. Reportedly, the aqueous leaf extract of MO decreased the viability of Jurkat cells, the immortalized line of human T lymphocytes, which are used to study acute T-cell leukemia [49]. The *in vitro* antiproliferative potential of MO extracts against leukemia, namely THP-1 human acute monocytic leukemia, HL-60 human acute promyelocytic leukemia, and K562 human chronic myelogenous leukemia, has been confirmed through SRB and MTT tetrazolium reduction assays [40].

Potestà et al. (2019) reported the antiproliferative and pro-apoptotic effects of various aqueous extracts derived from leaves and seeds of MO on lymphoid and

monocytoid cells not on peripheral blood mononuclear cells from healthy donors [62]. They concluded that this ability could be associated with the microRNA present in the extracts which highlighted their possible use as an adjuvant in traditional cancer therapy.

Dilworth et al. (2020) emphasized the antioxidative properties of MO leaf extract on markers of oxidative stress in HL60 cells, a human leukemia cell line, exposed to oxidative stress [65]. They showed that supplementation with the MO leaf extract at all concentrations resulted in significant reductions in lipid peroxidation in cells and increased cell viability with unknown mechanism.

Miscellaneous tumors

The antiproliferative effect of different fractions and the crude methanol extract of MO leaf were tested on the HeLa cell line. MO leaf extracts inhibited the proliferation of KB cells (a subline of the ubiquitous keratin-forming tumor cell line HeLa) through induction of apoptosis in a dose-dependent manner [30]. The ethanol-water [70-30] extracts of MO callus and leaf, significantly decreased the viability of HeLa cells in a dose-dependent manner. However, the leaf extract of MO appeared to be more effective compared to callus extract since the content of phenolic compounds present in leaf extract was higher than that of callus extract. Authors proposed that phenolic compounds are involved in the cytotoxicity triggered by MO [35]. Different cell lines including HeLa, were treated with the essential oil extracted from MO seeds at a range of concentrations from 0.15 to 1 mg/mL for 24h, and the cytotoxicity was assessed using MTT assay. Findings showed a significant reduction in cell viability in response to the ascending dose of essential oil in all cell lines [38]. The MO soluble cold distilled water extract, greatly induced apoptosis, inhibited tumor cell growth, and lowered the level of internal ROS in HT1080 human fibrosarcoma cell line [15]. The L929 cell line (subcutaneous connective tissue, areolar and adipose) was treated with the essential oil from MO seeds and a significantly reduced cell viability was reported in response to the ascending dose of essential oil in all

treated cell line [38]. Recently, bis (isothiocyanatomethyl) benzene, an anti-neoplastic phytochemical, has been purified from MO leaf extract which posed a suppression on cancer cell growth and inhibited carcinogenesis on HeLa cell line by caspase 3 apoptotic pathway in comparison to Cis-Platin [60].

Patriota et al. (2020) reported the antitumor activity of the MO flower trypsin inhibitor in sarcoma 180 inoculated mice [69]. They reported profound tumor regression and anti-angiogenesis without histological damages in other organs.

Lu et al. (2021) investigated the degradation of moringin (4-((α -L-rhamnosyloxy) benzyl)-isothiocyanate), a major bioactive isothiocyanate (ITC) found in MO seeds, at various food processing conditions [81]. They concluded that processing may lead to inactivation or perturbation of cytotoxicity of moringin against cancer cells.

Discussion

The MO is an omnipotent plant and almost all its parts have been used in various schools of traditional medicine and in culinary menus of south America, India, and Asia. This plant can be considered as an efficient tool for green synthesis of anticancer phyto-nanoparticles (for a review see Tiloke et al., 2018 [87]), nutraceutical source contains general (vitamin A, vitamin C, milk protein, and active phyto-constituents like alkaloids, protein, quinine, saponins, flavonoids, tannin, steroids, glycosides, fixed oil and fats) and special compounds like niazinin A, niazinin B and niazimicin A, niaziminin B [5] with a panaceas therapeutic power including anti-inflammatory, antioxidative, anti-cancerous, hepatoprotective, neuroprotective, hypoglycemic, and blood lipid-reducing functions [8], pharmaceutical source [6], agricultural resource for its cultivations and preparation of germplasm bank for assessing the reasons of its diversities in both morphology and compositions and even for biodiesel production [10] and more specifically oncotherapeutic and targeting chemopreventive properties of MO (for review see Karim et al., 2016; Khor et al., 2018 [4] [88]). In this regard, Khor and colleagues (2018) reported anti-cancer potentials of MO in a seminal review till 2018, however their review has not been covered all relevant studies and selection biases were obvious in presenting data [88]. It is worthy to mention, the numbers of studies focusing on anti-cancer properties of MO showed a surge after 2018 and to the best of my knowledge this is the first systematic review *inter alia*. More recently, Ercan et al. (2021) has reviewed antioxidative potential of MO and proposed it as an antiproliferative source [89].

Both anecdotal narratives belonging to various schools of traditional medicine and mainstream scientific evidence propose that MO-derived phytochemicals and botanical formulations have antitumor potential [48, 90]. The chemopreventive characteristics of MO have been extensively reported through a seminal review [4]; however, the mentioned review adopted a disintegrated approach and haphazardly examined general chemopreventive mechanisms, phytochemistry,

and pharmacognosy and cannot offer us a conclusion regarding oncotherapeutic or onco-prophylactic properties of MO although give us a good cue for focusing on this miracle tree. Accordingly, current systematic review, from 1998 to 2021, was implemented so as to obtain more scrutinized information (\neq knowledge) and gain further insight in order to put a conclusive end on \sim 24 years of research concerning the antitumor properties of MO. This area of research is very active and anybody looks over Table 1 will find that it is a great research of interest especially among Asian and Indian researchers, however these researchers just playing at the temporary field of *in vitro* (51% of studies) or *in vivo* chemically induced model (21% of studies) or tumor graft models (8% of studies; Figure 4) and there is no clinical studies in both medicine and veterinary medicine and it seems that there is courage to incite scientists to go from traditional medicine to bench but there is no encourage to leave bench to the destination of bed or human clinical studies. The reason of this stagnation in the *in vitro* or preclinical phase in this research avenue should be discussed in the cancer societies like Asian Clinical Oncology Society (asia-acos.org) and Indian Cancer Society (indiancancersociety.org) or their allies.

In this line, the higher proportion of works only reported therapeutic or preventive effects of MO extract and rarely specified phytochemicals mediating the antitumor effects except more recently investigations which have mechanistic insight to anti-tumor effects of MO in both gene and protein levels [75, 77]. To be more precise, Rhamno-Benzyl-GS and Ac-Isomer-GS III, quercetin-3-O-glucoside, 4-(β -D-glucopyranosyl-1 \rightarrow 4- α -L-rhamnopyranosyloxy)-benzyl isothiocyanate, and moringin, are remarkable MO phytochemicals having been reported to serve strong antitumor effects. Further scrutiny is urgently required to shed light upon the sphere of the unknown on which phytochemicals are the major bioactive compounds found in MO extract possessing potential antitumor effect against cancers. To the best of my knowledge, there is not any report in terms of antitumor activity of MO against metastatic cancers. Interestingly, a few studies have put their steps beyond the descriptive reports of antitumor effect of MO on colorectal cancers and introduced those phytochemicals promoting antitumor activities. Reportedly, both dietary fiber and phenolic compounds of MO leaves showed to impose suppressive effect on chemically-induced colorectal cancer. A fermented formulation of MO, established substantial anti-inflammatory effects and possibly may be involved in producing antitumor as well as antioxidative effects. A long way still exists to determine the profile of MO prebiotics found in various parts in both fermented and non-fermented formulations and this can be considered as a gap of knowledge in terms of MO pharmacognosy. In another seminal work, the inhibitory effect of MO leaf extract on human pancreatic cancer has been confirmed both alone and in combination with cisplatin. The number of studies that focused on synergistic effects of MO on the other canonical anti-neoplastic drugs and radiotherapeutics is very scarce.

All 21 review articles which targeted in this systematic review have been deeply analyzed technically to distillate their contents for discussing here. I should declare that the framework and backbone of them were heterogeneous and amalgam of traditional approaches mixed by orthodox medicine approaches covered ethnobotanical, pharmacognostic, agricultural and pharmacological investigations. Although this broad vision may reflect the miracle panacean properties of MO but cannot give us a cue to highlight the present and the future of this pipeline of research based on previous achievements. If I drew an analogy, reviewers just hit in outer ring of dartboard distant to high score rings or even bullseye. In nutshell, good samples of interest to understand general phytochemistry and therapeutic benefits of MO have been recently published (for a review see Arora and Arora, 2021; Hassan et al., 2021 [91, 92]).

As shown in Figure 5, leaves of MO as major botanical part (52%) have been used for its anticancer activities and since its decoction and boiled formulations are reported (Table 1) and there are some profound documented traditions [34] which convey prior-experiences of humankind regarding drinking of MO, it can be concluded that future market of functional tea of this plant would be at the top list of functional anticancer soft- or even hard-drinks. A simple and fast snapshot of “moringa tea” in Google image will show us the current market of functional products of MO, however based on my personal experience, fresh leaves would be more fragrant and aromatic and drying will diminish significantly the quality of hot and cold teas. It seems that employing of some conservation methods like cryopreservation would be strategic to hold natural flavor of moringa tea (for a review see Arora and Arora, 2021). After leaves, seeds of MO have been used to prepare various anti-cancer formulations including fresh or frozen aqueous extracts, coagulant or purified lectins, essential oil derived via cold pressing, and organic, enzyme-treated or defatted moringin-rich extract (Table 1). The MO seed would be considered as an oil seed source with high content of anticancer phytochemicals, however the more phytochemical and mechanistic investigations are requested to decipher its major phytochemical. Based on studies which analyzed in this systematic review (Table 1) moringin and its analogs would be considered as putative *phyto-onco-lytics* or *phyto-onco-statics* that I coined here arrogantly. In this line, approximately 7% of studies reported some anticancer phytochemicals derived from various parts of MO. In this context, more phytochemical and computational studies are requested to delve chemical spaces of *phyto-onco-lytics*, *phyto-onco-statics* or tumorigenic, proto-oncogenic, and proangiogenic compounds of MO. For instance, Fernandes et al. (2016) warned us to survey the effect of MO extracts or its phytochemicals on angiogenesis before considering them as prospective candidates for antitumor drug [43].

Pods would be considered as an initial guard of seeds and their nutritional values are very different among cultivars and ecotypes (for a review see Arora and Arora, 2021). Many tribes cook pods and use them as food,

however 7% of reported studies in this systematic review mentioned to their anticancer activities (Table 1) and moringin has been introduced as their main functional components. The phytochemistry and deciphering functional components of the powders of mature and immature pods may be an unknown features of MO which requests more scientific investigations. The contributions of other parts of MO including callus, bark, root, fruit pulp and flowers were less than 5 percentage of studies and would be investigated in the future (Table 1). In addition, it is worthy to note the partitioning of secondary metabolites with pharmacological values in source (leaf) and sink (roots, stems, pods, callus, and fruits) organs of MO for selecting the best solvents for their extractions. The secondary metabolites usually produced in plants exposed to a plethora of (a) biotic stresses therefore it is possible to manipulate MO to decipher enriching ecotypes or to concentrate high amount of *phyto-oncolytics* or *phyto-oncostatics* or engineer new cultivars by using biopharming and transgenic tools.

In this continuum, various techniques of extractions including maceration, decoction, percolation, cold pressing, etc. have been reported in some selected studies and it seems that rational extractions have not been pursued in all studies. The various physicochemical attributes of extracts and the phytochemical of interest dictate us to select the best solvents for extraction or fractionation of a part of MO to reach the most potent formulations or even phytochemicals (for a review see Abubakar et al., 2020). As shown in Figure 6, solvents commonly used in extraction of MO are polar solvent (e.g., water, ethanol, hydro-alcohol, methanol), intermediate polar (e.g., acetone for fractionation, dichloromethane), and nonpolar (e.g., n-hexane, ethyl acetate, chloroform). Some studies (8%) have been employed lyophilization as a new method to break cell walls and to extract its content by using appropriate solvents. Among the solvents have been utilized to extract or to fractionate (1% of studies) resulting extracts of MO, polar solvents had contributions in this order: water (29%) > ethanol (17%) > methanol (13%) > hydro-alcohol (11%) and the rest of intermediate polar (dichloromethane 4%) and nonpolar (n-hexane (7%), ethyl acetate (7%), chloroform (3%)) solvents. To sum up, future studies must be focused on the rational extraction and purification of MO with special thanks to nonpolar solvents or phytochemical and pharmacological features of menstruum and marc.

As shown in Figure 7, human colorectal cancer was the main tumor phenotype which was studied among other phenotypes and consisted 22% of screened studies. Leukemia, non-small cell adenocarcinoma, and breast cancer consisted 14, 10, and 8% of screened studies, respectively and the rest of tumors consisted less than 5% of studies. To sum up, various extracts of MO have been showed *phyto-onco-lytic* and/or *phyto-onco-static* activities against a wide ranges of tumor phenotypes including benign, malignant, and metastatic ones. Figure 7 gives us a research cue to test antitumor activity of MO in other tumors, to select specific phenotype for *in vivo* animal model or even to try clinical trial for some

mentioned phenotypes in medicine or veterinary medicine.

As depicted in Figure 8 in a radar format, totally 118 cell lines have been used to detect antitumor activity of various extracts of MO and in this context, healthy cell lines (control; n = 19), MCF-7 (n = 12), HepG2 (n = 12), and Hela (n = 6) consisted top list cell lines employed in various studies. This radar format gives us an opportunity to select new cell lines for novel works to understand broad spectrum of antitumor activity of MO and it also can give us the highly responsive cell lines to dig mechanisms of MO antitumor activities. Young researchers can curate more information regarding disease, disease subtype, lineages, source, gender, and primary/metastasis of all mentioned cell lines in depmap (<https://depmap.org/portal/depmap/>).

The major anti-cancer portals of MO which have been classified in Figure 9 may be considered redundant however they are distilled from selected studies of this systematic review via both text mining and semantics. In this scenario, the summation of all anti-cancer portals was 76 for all selected papers. The induction of apoptosis (n = 29), anti-proliferation (n = 17), anti-angiogenesis (n = 8), and DNA/RNA fragmentation (n = 6) were the main portals mediated antitumor effects of MO. The cytotoxicity and anti-inflammation may be considered as minor antitumor portals. The difference between the number of selected studies of this systematic review (n = 71) and total number of reported antitumor portals (n = 76) is due to report of more than one determinant outcomes per each study.

In conclusion, future studies must be focused on the rational extraction and purification of MO, phytochemistry, and computational and experimental pharmacology of various extracts of MO to decipher *phyto-onco-lytic* and/or *phyto-onco-static* drug-like phytochemicals and the necessity of starting clinical trials should be discussed and pursued especially for tumors which have sufficient preclinical evidence. In this line, I am focusing on a hybrid study (computational plus experimental) to decipher pharmacological mechanism of anti-breast cancer activities of MO.

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References

- Weber D. Botanical Oncology; isolates. Sydney: Panacea Publishing 2015.
- Sood A., Prasad K. (2010) Challenges Conducting Clinical Trials with Herbal Products in Oncology. In: Alaoui-Jamali M. (eds) Alternative and Complementary Therapies for Cancer. Springer, Boston, MA.
- Wang H, Khor TO, Shu L, Su Z, Fuentes F, Lee J, Kong AT. Plants vs. cancer: a review on natural phytochemicals in preventing and treating cancers and their druggability. *Anti-Cancer Agents in Medicinal Chemistry*. 2012 Dec;12(10):1281-1305. <https://doi.org/10.2174/187152012803833026>
- Karim NA, Ibrahim MD, Kntayya SB, Rukayadi Y, Hamid HA, Razis AF. *Moringa oleifera* Lam: targeting chemoprevention. *Asian Pac J Cancer Prev*. 2016;17(8):3675-86.
- Abdull Razis AF, Ibrahim MD, Kntayya SB. Health benefits of *Moringa oleifera*. *Asian Pacific journal of cancer prevention: APJCP*. 2014;15(20):8571-8576. <https://doi.org/10.7314/apjcp.2014.15.20.71>
- Paikra BK, Dhongade HKJ, Gidwani B. Phytochemistry and Pharmacology of *Moringa oleifera* Lam. *Journal of Pharmacopuncture*. 2017 09;20(3):194-200. <https://doi.org/10.3831/KPI.2017.20.022>
- Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive Components in *Moringa Oleifera* Leaves Protect against Chronic Disease. *Antioxidants (Basel, Switzerland)*. 2017 Nov 16;6(4):E91. <https://doi.org/10.3390/antiox6040091>
- Kou X, Li B, Olayanju JB, Drake JM, Chen N. Nutraceutical or Pharmacological Potential of *Moringa oleifera* Lam. *Nutrients*. 2018 03 12;10(3):E343. <https://doi.org/10.3390/nu10030343>
- Hussain S, Malik F, Mahmood S. Review: an exposition of medicinal preponderance of *Moringa oleifera* (Lank.). *Pak J Pharm Sci*. 2014 Mar;27(2):397-403.
- Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. Cultivation, Genetic, Ethnopharmacology, Phytochemistry and Pharmacology of *Moringa oleifera* Leaves: An Overview. *International Journal of Molecular Sciences*. 2015 06 05;16(6):12791-12835. <https://doi.org/10.3390/ijms160612791>
- Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ*. 2009 07 21;339:b2700. <https://doi.org/10.1136/bmj.b2700>
- Guevara AP, Vargas C, Sakurai H, Fujiwara Y, Hashimoto K, Maoka T, Kozuka M, Ito Y, Tokuda H, Nishino H. An antitumor promoter from *Moringa oleifera* Lam. *Mutation Research*. 1999 04 06;440(2):181-188. [https://doi.org/10.1016/s1383-5718\(99\)00025-x](https://doi.org/10.1016/s1383-5718(99)00025-x)
- Bharali R, Tabassum J, Azad MR. Chemomodulatory effect of *Moringa oleifera*, Lam, on hepatic carcinogen metabolising enzymes, antioxidant parameters and skin papillomagenesis in mice. *Asian Pac J Cancer Prev*. 2003 Apr-Jun;4(2):131-9...
- Gismondi A, Canuti L, Impei S, Di Marco G, Kenzo M, Colizzi V, Canini A. Antioxidant extracts of African medicinal plants induce cell cycle arrest and differentiation in B16F10 melanoma cells. *International Journal of Oncology*. 2013 09;43(3):956-964. <https://doi.org/10.3892/ijo.2013.2001>
- Jung IL. Soluble extract from *Moringa oleifera* leaves with a new anticancer activity. *PloS One*. 2014;9(4):e95492. <https://doi.org/10.1371/journal.pone.0095492>
- Andrade Luz L, Rossato FA, Costa RAPE, Napoleão TH,

- Paiva PMG, Coelho LCBB. Cytotoxicity of the coagulant *Moringa oleifera* lectin (cMoL) to B16-F10 melanoma cells. *Toxicology in vitro: an international journal published in association with BIBRA*. 2017 Oct;44:94-99. <https://doi.org/10.1016/j.tiv.2017.06.019>
17. Do BH, Hoang NS, Nguyen TPT, Ho NQC, Le TL, Doan CC. Phenolic Extraction of *Moringa Oleifera* Leaves Induces Caspase-Dependent and Caspase-Independent Apoptosis through the Generation of Reactive Oxygen Species and the Activation of Intrinsic Mitochondrial Pathway in Human Melanoma Cells. *Nutrition and Cancer*. 2021;73(5):869-888. <https://doi.org/10.1080/01635581.2020.1776885>
 18. Do BH, Hoang NS, Nguyen TPT, Ho NQC, Le TL, Doan CC. Phenolic extraction of *MoringaOleifera* leaves induces caspase-dependent and caspase-independent apoptosis through the generation of reactive oxygen species and the activation of intrinsic mitochondrial pathway in human melanoma cells. *Nutr Cancer*. 2021;73(5):869-888. doi: 10.1080/01635581.2020.1776885. Epub 2020 Jun 12. PMID: 32530312.
 19. Luetragoon T, Pankla Sranujit R, Noysang C, Thongsri Y, Potup P, Suphrom N, Nuengchamnong N, Usuwanthim K. Anti-Cancer Effect of 3-Hydroxy- β -Ionone Identified from *Moringa oleifera* Lam. Leaf on Human Squamous Cell Carcinoma 15 Cell Line. *Molecules (Basel, Switzerland)*. 2020 08 05;25(16):E3563. <https://doi.org/10.3390/molecules25163563>
 20. Khalafalla MM, Abdellatef E, Dafalla HM, Nassrallah AA, Aboul-Encin KM, Lightfoot DA, El-Deeb FE, El-Shemy HA. Active principle from *Moringa oleifera* Lam leaves effective against two leukemias and a hepatocarcinoma, *African Journal of Biotechnology*, 2010, 9, 8467-8471.
 21. Budda S, Butryee C, Tuntipopipat S, Rungsipipat A, Wangnaithum S, Lee JS, Kupradinun P. Suppressing effects of *Moringa oleifera* Lam pod against mouse colon carcinogenesis induced by azoxymethane and dextran sodium sulfate. *Asian Pac J Cancer Prev*. 2011;12(12):3221-8.
 22. Pamok S, Saenphet S, Saenphet K. Antiproliferative effect of *Moringa oleifera* Lam. and *Pseuderanthemum palatiferum* (Nees) Radlk extracts on the colon cancer cells. *Journal of medicinal plant research*. 2012 01 09;6:139-145. <https://doi.org/10.5897/JMPR11.1343>
 23. Sharma V, Paliwal R, Janmeda P, Sharma S. Chemopreventive efficacy of *Moringa oleifera* pods against 7, 12-dimethylbenz[a]anthracene induced hepatic carcinogenesis in mice. *Asian Pacific journal of cancer prevention: APJCP*. 2012;13(6):2563-2569. <https://doi.org/10.7314/apjcp.2012.13.6.2563>
 24. Waiyaput W, Payungporn S, Issara-Amphorn J, Panjaworayan NT. Inhibitory effects of crude extracts from some edible Thai plants against replication of hepatitis B virus and human liver cancer cells. *BMC complementary and alternative medicine*. 2012 Dec 06;12:246. <https://doi.org/10.1186/1472-6882-12-246>
 25. Berkovich L, Earon G, Ron I, Rimmon A, Vexler A, Lev-Ari S. *Moringa Oleifera* aqueous leaf extract down-regulates nuclear factor-kappaB and increases cytotoxic effect of chemotherapy in pancreatic cancer cells. *BMC complementary and alternative medicine*. 2013 08 19;13:212. <https://doi.org/10.1186/1472-6882-13-212>
 26. Murakami A, Kitazono Y, Jiwajinda S, Koshimizu K, Ohigashi H. Niaziminin, a thiocarbamate from the leaves of *Moringa oleifera*, holds a strict structural requirement for inhibition of tumor-promoter-induced Epstein-Barr virus activation. *Planta Medica*. 1998 05;64(4):319-323. <https://doi.org/10.1055/s-2006-957442>
 27. Promkum C, Kupradinun P, Tuntipopipat S, Butryee C. Nutritive evaluation and effect of *Moringa oleifera* pod on clastogenic potential in the mouse. *Asian Pac J Cancer Prev*. 2010;11(3):627-32.
 28. Brunelli D, Tavecchio M, Falcioni C, Frapolli R, Erba E, Iori R, Rollin P, Barillari J, Manzotti C, Morazzoni P, D'Incalci M. The isothiocyanate produced from glucomoringin inhibits NF-kB and reduces myeloma growth in nude mice *in vivo*. *Biochemical Pharmacology*. 2010 04 15;79(8):1141-1148. <https://doi.org/10.1016/j.bcp.2009.12.008>
 29. Paliwal R, Sharma V, Pracheta A, Sharma S, Yadav S and Sharma S: Anti-nephrotoxic effect of administration of *Moringa oleifera* Lam in amelioration of DMBA-induced renal carcinogenesis in Swiss albino mice. *Biol Med Special Issue 3: 27-35*, 2011.
 30. Sreelatha S, Jeyachitra A, Padma PR. Antiproliferation and induction of apoptosis by *Moringa oleifera* leaf extract on human cancer cells. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*. 2011 06;49(6):1270-1275. <https://doi.org/10.1016/j.fct.2011.03.006>
 31. Tiloke C, Phulukdaree A, Chuturgoon AA. The antiproliferative effect of *Moringa oleifera* crude aqueous leaf extract on cancerous human alveolar epithelial cells. *BMC complementary and alternative medicine*. 2013 09 16;13:226. <https://doi.org/10.1186/1472-6882-13-226>
 32. Araújo LCC, Aguiar JS, Napoleão TH, Mota FVB, Barros ALS, Moura MC, Coriolano M, Coelho LCBB, Silva TG, Paiva PMG. Evaluation of Cytotoxic and Anti-Inflammatory Activities of Extracts and Lectins from *Moringa oleifera* Seeds. *PLoS ONE*. 2013 Dec 09;8(12):e81973. <https://doi.org/10.1371/journal.pone.0081973>
 33. Ghosh N. Anticancer effect of *Moringa oleifera* leaf extract on human breast cancer cell. Thesis submitted to Jadavpur University Faculty of Engineering and Technology School of Bioscience & Engineering. Kolkata – 700 032, India. 2014.
 34. Charoensin S. Antioxidant and anticancer activities of *Moringa oleifera* leaves. *Journal of Medicinal Plants Research*. 2014 02 17;8:318-325. <https://doi.org/10.5897/JMPR2013.5353>
 35. Jafarain A, Asghari G, Ghassami E. Evaluation of cytotoxicity of *Moringa oleifera* Lam. callus and leaf extracts on HeLa cells. *Advanced Biomedical Research*. 2014;3:194. <https://doi.org/10.4103/2277-9175.140668>
 36. Akanni EO, Adedeji AL, Adedosu OT, Olaniran OI, Oloke JK. Chemopreventive and anti-leukemic effects of ethanol extracts of *Moringa oleifera* leaves on wistar rats bearing benzene induced leukemia. *Current Pharmaceutical Biotechnology*. 2014;15(6):563-568. <https://doi.org/10.2174/1389201015666140717090755>
 37. Leelawat S and Leelawat K: *Moringa oleifera* extracts induce cholangiocarcinoma cell apoptosis by induction of reactive oxygen species production. *Int J Pharmacog Phytochem Res* 6: 183-189, 2014.
 38. Elsayed EA, Sharaf-Eldin MA, Wadaan M. *In vitro* Evaluation of Cytotoxic Activities of Essential Oil from *Moringa oleifera* Seeds on HeLa, HepG2, MCF-7, CACO-2 and L929 Cell Lines. *Asian Pacific journal of cancer prevention: APJCP*. 2015;16(11):4671-4675. <https://doi.org/10.7314/apjcp.2015.16.11.4671>
 39. Al-Asmari AK, Albalawi SM, Athar MT, Khan AQ, Al-Shahrani H, Islam M. *Moringa oleifera* as an Anti-Cancer Agent against Breast and Colorectal Cancer Cell Lines. *PloS One*. 2015;10(8):e0135814. <https://doi.org/10.1371/journal.pone.0135814>
 40. Diab KAE, Guru SK, Bhushan S, Saxena AK. *In Vitro* Anticancer Activities of *Anogeissus latifolia*, *Terminalia*

- bellerica, Acacia catechu and *Moringa oleifera* Indian Plants. Asian Pacific journal of cancer prevention: APJCP. 2015;16(15):6423-6428. <https://doi.org/10.7314/apjcp.2015.16.15.6423>
41. Jung IL, Lee JH, Kang SC. A potential oral anticancer drug candidate, *Moringa oleifera* leaf extract, induces the apoptosis of human hepatocellular carcinoma cells. *Oncology Letters*. 2015 09;10(3):1597-1604. <https://doi.org/10.3892/ol.2015.3482>
 42. Krishnamurthy PT, Vardarajulu A, Wadhvani A, Patel V. Identification and characterization of a potent anticancer fraction from the leaf extracts of *Moringa oleifera* L. *Indian J Exp Biol*. 2015 Feb;53(2):98-103.
 43. Fernandes EE, Pulwale AV, Patil GA, Moghe AS. Probing Regenerative Potential of *Moringa oleifera* Aqueous Extracts Using *In vitro* Cellular Assays. *Pharmacognosy Research*. 2016 Dec;8(4):231-237. <https://doi.org/10.4103/0974-8490.188877>
 44. Maiyo FC, Moodley R, Singh M. Cytotoxicity, Antioxidant and Apoptosis Studies of Quercetin-3-O Glucoside and 4-(β-D-Glucopyranosyl)-1→4-α-L-Rhamnopyranosyloxy)-Benzyl Isothiocyanate from *Moringa oleifera*. *Anti-Cancer Agents in Medicinal Chemistry*. 2016;16(5):648-656. <https://doi.org/10.2174/1871520615666151002110424>
 45. Rajan TS, De Nicola GR, Iori R, Rollin P, Bramanti P, Mazzon E. Anticancer activity of glucomoringin isothiocyanate in human malignant astrocytoma cells. *Fitoterapia*. 2016 04;110:1-7. <https://doi.org/10.1016/j.fitote.2016.02.007>
 46. Tiloke C, Phulukdaree A, Chuturgoon AA. The Antiproliferative Effect of *Moringa oleifera* Crude Aqueous Leaf Extract on Human Esophageal Cancer Cells. *Journal of Medicinal Food*. 2016 04;19(4):398-403. <https://doi.org/10.1089/jmf.2015.0113>
 47. Michl C, Vivarelli F, Weigl J, De Nicola G, Canistro D, Paolini M, et al. The chemopreventive phytochemical moringin isolated from *Moringa oleifera* seeds inhibits JAK/STAT signaling. *PLoS One*. 2016;11(6):e0157430. <https://doi.org/10.1371/journal.pone.0157430>
 48. Förster N, Mewis I, Glatt H, Haack M, Brigelius-Flohé R, Schreiner M, Ulrichs C. Characteristic single glucosinolates from *Moringa oleifera*: Induction of detoxifying enzymes and lack of genotoxic activity in various model systems. *Food & Function*. 2016 Nov 09;7(11):4660-4674. <https://doi.org/10.1039/c6fo01231k>
 49. Madi N, Dany M, Abdoun S, Usta J. *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*. 2016 Oct;35(7):604-613. <https://doi.org/10.1080/07315724.2015.1080128>
 50. Cuellar-Núñez ML, Luzardo-Ocampo I, Campos-Vega R, Gallegos-Corona MA, González de Mejía E, Loarca-Piña G. Physicochemical and nutraceutical properties of moringa (*Moringa oleifera*) leaves and their effects in an *in vivo* AOM/DSS-induced colorectal carcinogenesis model. *Food Research International*. 2018 03;105:159-168. <https://doi.org/10.1016/j.foodres.2017.11.004>
 51. Sadek KM, Abouzed TK, Abouelkhair R, Nasr S. The chemo-prophylactic efficacy of an ethanol *Moringa oleifera* leaf extract against hepatocellular carcinoma in rats. *Pharmaceutical Biology*. 2017 Dec;55(1):1458-1466. <https://doi.org/10.1080/13880209.2017.1306713>
 52. Tragulpakseerojn J, Yamaguchi N, Pamonsinlapatham P, Wetwitayaklung P, Yoneyama T, Ishikawa N, Ishibashi M, Apirakaramwong A. Anti-proliferative effect of *Moringa oleifera* Lam (Moringaceae) leaf extract on human colon cancer HCT116 cell line. *Tropical Journal of Pharmaceutical Research*. 2017 03 07;16(2):371-378. <https://doi.org/10.4314/tjpr.v16i2>
 53. Leelawat S, Leelawat K. Molecular mechanisms of cholangiocarcinoma cell inhibition by medicinal plants. *Oncology Letters*. 2017 02;13(2):961-966. <https://doi.org/10.3892/ol.2016.5488>
 54. Adebayo IA, Arsad H, Samian MR. Antiproliferative effect on breast cancer (MCF7) of *Moringa oleifera* seed extracts. *African journal of traditional, complementary, and alternative medicines: AJTCAM*. 2017;14(2):282-287. <https://doi.org/10.21010/ajtcam.v14i2.30>
 55. Giacoppo S, Iori R, Bramanti P, Mazzon E. Moringa isothiocyanate complexed with α-cyclodextrin: a new perspective in neuroblastoma treatment. *BMC complementary and alternative medicine*. 2017 07 14;17(1):362. <https://doi.org/10.1186/s12906-017-1876-z>
 56. Antonini E, Iori R, Ninfali P, Scarpa ES. A Combination of Moringin and Avenanthramide 2f Inhibits the Proliferation of Hep3B Liver Cancer Cells Inducing Intrinsic and Extrinsic Apoptosis. *Nutrition and Cancer*. 2018 Oct;70(7):1159-1165. <https://doi.org/10.1080/01635581.2018.1497672>
 57. Asadzaman AKM, Hasan I, Chakraborty A, Zaman S, Islam SS, Ahmed FRS, Kabir KMA, Nurujjaman M, Uddin MB, Alam MT, Shaha RK, Kabir SR. *Moringa oleifera* seed lectin inhibits Ehrlich ascites carcinoma cell growth by inducing apoptosis through the regulation of Bak and NF-κB gene expression. *International Journal of Biological Macromolecules*. 2018 02;107(Pt B):1936-1944. <https://doi.org/10.1016/j.ijbiomac.2017.10.070>
 58. Jaafaru MS, Nordin N, Shaari K, Rosli R, Abdull Razis AF. Isothiocyanate from *Moringa oleifera* seeds mitigates hydrogen peroxide-induced cytotoxicity and preserved morphological features of human neuronal cells. *PLoS One*. 2018;13(5):e0196403. <https://doi.org/10.1371/journal.pone.0196403>
 59. Hagoel L, Vexler A, Kalich-Philosoph L, Earon G, Ron I, Shtabsky A, Marmor S, Lev-Ari S. Combined Effect of *Moringa oleifera* and Ionizing Radiation on Survival and Metastatic Activity of Pancreatic Cancer Cells. *Integrative Cancer Therapies*. 2019 Dec;18:1534735419828829. <https://doi.org/10.1177/1534735419828829>
 60. Paul S, Basak P, Maity N, Guha C, Jana NK. Bis (Isothiocyanatomethyl) Benzene, A Plant Derived Anti-Neoplastic Compound: Purified from *Moringa Oleifera* Leaf Extract. *Anti-Cancer Agents in Medicinal Chemistry*. 2019;19(5):677-686. <https://doi.org/10.2174/187152061966190206164137>
 61. Kumar G, Gupta R, Sharan S, Roy P, Pandey DM. Anticancer activity of plant leaves extract collected from a tribal region of India. *3 Biotech*. 2019 Nov;9(11):399. <https://doi.org/10.1007/s13205-019-1927-x>
 62. Potestà M, Minutolo A, Gismondi A, Canuti L, Kenzo M, Roglia V, Macchi F, Grelli S, Canini A, Colizzi V, Montesano C. Cytotoxic and apoptotic effects of different extracts of *Moringa oleifera* Lam on lymphoid and monocytoid cells. *Experimental and Therapeutic Medicine*. 2019 07;18(1):5-17. <https://doi.org/10.3892/etm.2019.7544>
 63. Tiloke C, Phulukdaree A, Gengan RM, Chuturgoon AA. *Moringa oleifera* Aqueous Leaf Extract Induces Cell-Cycle Arrest and Apoptosis in Human Liver Hepatocellular Carcinoma Cells. *Nutrition and Cancer*. 2019;71(7):1165-1174. <https://doi.org/10.1080/01635581.2019.1597136>
 64. Khan F, Pandey P, Ahmad V, Upadhyay TK. *Moringa oleifera* methanolic leaves extract induces apoptosis and G0/G1 cell cycle arrest via downregulation of Hedgehog Signaling Pathway in human prostate PC-3 cancer cells. *Journal of Food Biochemistry*. 2020 08;44(8):e13338. <https://doi.org/10.1080/01635581.2019.1597136>

- org/10.1111/jfbc.13338
65. Dilworth LL, Stennett D, Omoruyi FO. Effects of *Moringa oleifera* Leaf Extract on Human Promyelocytic Leukemia Cells Subjected to Oxidative Stress. *Journal of Medicinal Food*. 2020 07;23(7):728-734. <https://doi.org/10.1089/jmf.2019.0192>
 66. Xie J, Luo F, Shi C, Jiang W, Qian Y, Yang M, Song S, Dai T, Peng L, Gao X, Tao L, Tian Y, Sheng J. *Moringa oleifera* Alkaloids Inhibited PC3 Cells Growth and Migration Through the COX-2 Mediated Wnt/ β -Catenin Signaling Pathway. *Frontiers in Pharmacology*. 2020;11. <https://doi.org/10.3389/fphar.2020.523962>
 67. Aldakheel RK, Rehman S, Almessiere MA, Khan FA, Gondal MA, Mostafa A, Baykal A. Bactericidal and *In Vitro* Cytotoxicity of *Moringa oleifera* Seed Extract and Its Elemental Analysis Using Laser-Induced Breakdown Spectroscopy. *Pharmaceuticals (Basel, Switzerland)*. 2020 08 13;13(8):E193. <https://doi.org/10.3390/ph13080193>
 68. Phannasil P, Roytrakul S, Phaonakrop N, Kupradinun P, Budda S, Butryee C, Akekawatchai C, Tuntipopipat S. Protein expression profiles that underpin the preventive and therapeutic potential of *Moringa oleifera* Lam against azoxymethane and dextran sodium sulfate-induced mouse colon carcinogenesis. *Oncology Letters*. 2020 08;20(2):1792-1802. <https://doi.org/10.3892/ol.2020.11730>
 69. Patriota LLDS, Ramos DDBM, Dos Santos ACLA, Silva YA, Gama E Silva M, Torres DJL, Procópio TF, Oliveira AM, Coelho LCBB, Pontual EV, Silva DCN, Paiva PMG, Lorena VMB, Mendes RL, Napoleão TH. Antitumor activity of *Moringa oleifera* (drumstick tree) flower trypsin inhibitor (MoFTI) in sarcoma 180-bearing mice. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association*. 2020 Nov;145:111691. <https://doi.org/10.1016/j.fct.2020.111691>
 70. Wisitpongpun P, Suphrom N, Potup P, Nuengchamngong N, Calder PC, Usuwanthim K. *In Vitro* Bioassay-Guided Identification of Anticancer Properties from *Moringa oleifera* Lam. Leaf against the MDA-MB-231 Cell Line. *Pharmaceuticals (Basel, Switzerland)*. 2020 Dec 15;13(12):E464. <https://doi.org/10.3390/ph13120464>
 71. Lim WF, Mohamad Yusof MI, Teh LK, Salleh MZ. Significant Decreased Expressions of CaN, VEGF, SLC39A6 and SFRP1 in MDA-MB-231 Xenograft Breast Tumor Mice Treated with *Moringa oleifera* Leaves and Seed Residue (MOLSr) Extracts. *Nutrients*. 2020 09 30;12(10):E2993. <https://doi.org/10.3390/nu12102993>
 72. Pandey P, Khan F. Jab1 Inhibition by Methanolic Extract of *Moringa Oleifera* Leaves in Cervical Cancer Cells: A Potent Targeted Therapeutic Approach. *Nutrition and Cancer*. 2021 Dec 31;73(11-12):2411-2419. <https://doi.org/10.1080/01635581.2020.1826989>
 73. Barhoi D, Upadhaya P, Barbhuiya SN, Giri A, Giri S. Aqueous Extract of *Moringa oleifera* Exhibit Potential Anticancer Activity and can be Used as a Possible Cancer Therapeutic Agent: A Study Involving *In Vitro* and *In Vivo* Approach. *Journal of the American College of Nutrition*. 2021 01;40(1):70-85. <https://doi.org/10.1080/07315724.2020.1735572>
 74. Fuel M, Mesas C, Martínez R, Ortiz R, Quiñonero F, Prados J, Porres JM, Melguizo C. Antioxidant and antiproliferative potential of ethanolic extracts from *Moringa oleifera*, *Tropaeolum tuberosum* and *Annona cherimola* in colorectal cancer cells. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2021 Nov;143:112248. <https://doi.org/10.1016/j.biopha.2021.112248>
 75. Siddiqui S, Upadhyay S, Ahmad I, Hussain A, Ahamed M. Cytotoxicity of *Moringa oleifera* fruits on human liver cancer and molecular docking analysis of bioactive constituents against caspase-3 enzyme. *Journal of Food Biochemistry*. 2021 05;45(5):e13720. <https://doi.org/10.1111/jfbc.13720>
 76. Zunica ERM, Yang S, Coulter A, White C, Kirwan JP, Gilmore LA. *Moringa Oleifera* Seed Extract Concomitantly Supplemented with Chemotherapy Worsens Tumor Progression in Mice with Triple Negative Breast Cancer and Obesity. *Nutrients*. 2021 08 24;13(9):2923. <https://doi.org/10.3390/nu13092923>
 77. Cuellar-Núñez ML, Gonzalez de Mejia E, Loarca-Piña G. *Moringa oleifera* leaves alleviated inflammation through downregulation of IL-2, IL-6, and TNF- α in a colitis-associated colorectal cancer model. *Food Research International (Ottawa, Ont.)*. 2021 06;144:110318. <https://doi.org/10.1016/j.foodres.2021.110318>
 78. Xie J, Peng L, Yang M, Jiang W, Mao J, Shi C, Tian Y, Sheng J. Alkaloid Extract of *Moringa oleifera* Lam. Exerts Antitumor Activity in Human Non-Small-Cell Lung Cancer via Modulation of the JAK2/STAT3 Signaling Pathway. *Evidence-Based Complementary and Alternative Medicine: eCAM*. 2021;2021:5591687. <https://doi.org/10.1155/2021/5591687>
 79. Mohd Fisall UF, Ismail NZ, Adebayo IA, Arsal H. Dichloromethane fraction of *Moringa oleifera* leaf methanolic extract selectively inhibits breast cancer cells (MCF7) by induction of apoptosis via upregulation of Bax, p53 and caspase 8 expressions. *Molecular Biology Reports*. 2021 05;48(5):4465-4475. <https://doi.org/10.1007/s11033-021-06466-y>
 80. Caicedo-Lopez LH, Cuellar-Núñez ML, Luzardo-Ocampo I, Campos-Vega R, Lóarca-Piña G. Colonic metabolites from digested *Moringa oleifera* leaves induced HT-29 cell death via apoptosis, necrosis, and autophagy. *International Journal of Food Sciences and Nutrition*. 2021 06;72(4):485-498. <https://doi.org/10.1080/09637486.2020.1849039>
 81. Lu Y, Maria Vos RD, Zhang Y, Zhang M, Liu Y, Fu C, Liu SQ, Huang D. The degradation kinetics and mechanism of moringin in aqueous solution and the cytotoxicity of degraded products. *Food Chemistry*. 2021 Dec 01;364:130424. <https://doi.org/10.1016/j.foodchem.2021.130424>
 82. Bhadresha KP., Jain NK., Rawal R.M. Assessing the Protective Effect of *Moringa oleifera* Extract against Bone Metastasis: An *In Vitro* Simulated Digestion Approach. *Nutrition and Cancer*, 2021; 1–14. <https://doi.org/10.1080/01635581.2021.1933099>
 83. Joung H, Kim B, Park H, Lee K, Kim H, Sim H, Do H, Hyun C, Do M. Fermented *Moringa oleifera* Decreases Hepatic Adiposity and Ameliorates Glucose Intolerance in High-Fat Diet-Induced Obese Mice. *Journal of Medicinal Food*. 2017 05;20(5):439-447. <https://doi.org/10.1089/jmf.2016.3860>
 84. Jaafaru MS, Abd Karim NA, Mohamed Eliaser E, Maitalata Waziri P, Ahmed H, Mustapha Barau M, Kong L, Abdull Razis AF. Nontoxic Glucomoringin-Isothiocyanate (GMG-ITC) Rich Soluble Extract Induces Apoptosis and Inhibits Proliferation of Human Prostate Adenocarcinoma Cells (PC-3). *Nutrients*. 2018 08 27;10(9):E1174. <https://doi.org/10.3390/nu10091174>
 85. Wright RJ, Lee KS, Hyacinth HI, Hibbert JM, Reid ME, Wheatley AO, Asemota HN. An Investigation of the Antioxidant Capacity in Extracts from *Moringa oleifera* Plants Grown in Jamaica. *Plants (Basel, Switzerland)*. 2017 Oct 23;6(4):E48. <https://doi.org/10.3390/plants6040048>
 86. Kalkunte S, Swamy N, Dizon DS, Brard L. Benzyl isothiocyanate (BITC) induces apoptosis in ovarian cancer cells *in vitro*. *J Exp Ther Oncol*. 2006;5(4):287-300.
 87. Tiloke C, Anand K, Gengan RM, Chuturgoon AA. *Moringa oleifera* and their phytonanoparticles: Potential

- antiproliferative agents against cancer. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2018 Dec;108:457-466. <https://doi.org/10.1016/j.biopha.2018.09.060>
88. Khor KZ, Lim V, Moses EJ, Abdul Samad N. The *In Vitro* and *In Vivo* Anticancer Properties of *Moringa oleifera*. *Evidence-Based Complementary and Alternative Medicine: eCAM*. 2018;2018:1071243. <https://doi.org/10.1155/2018/1071243>
89. Ercan K, Geceseffa OF, Taysi ME, Ali Ali OA, Taysi S. *Moringa Oleifera*: A Review of Its Occurrence, Pharmacological Importance and Oxidative Stress. *Mini Reviews in Medicinal Chemistry*. 2021;21(3):380-396. <https://doi.org/10.2174/1389557520999200728162453>
90. Popoola JO, Obembe OO. Local knowledge, use pattern and geographical distribution of *Moringa oleifera* Lam. (Moringaceae) in Nigeria. *Journal of Ethnopharmacology*. 2013 Nov 25;150(2):682-691. <https://doi.org/10.1016/j.jep.2013.09.043>
91. Arora S, Arora S. Nutritional significance and therapeutic potential of *Moringa oleifera*: The wonder plant. *Journal of Food Biochemistry*. 2021 Oct;45(10):e13933. <https://doi.org/10.1111/jfbc.13933>
92. Hassan MA, Xu T, Tian Y, Zhong Y, Ali FAZ, Yang X, Lu B. Health benefits and phenolic compounds of *Moringa oleifera* leaves: A comprehensive review. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*. 2021 Dec;93:153771. <https://doi.org/10.1016/j.phymed.2021.153771>
93. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. *Journal of Pharmacy & Bioallied Sciences*. 2020 03;12(1):1-10. https://doi.org/10.4103/jpbs.JPBS_175_19



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