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RESEARCH **Open Access**

Moringa oleifera as Anticancer: A Review of Recent Studies

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Abstract
Moringa oleifera Lam (MO) plants have long been reported to have many pharmacotherapy benefits. In vitro and in vivo studies have shown that MO extracts have various biological activities and therapeutic effects, including cardioprotective, cardiometabolic, hypocholesterolemic, neuroprotective, anti-inflammatory, antioxidant, anti-hypertensive, anti-diabetic, anti-bacterial, immunomodulatory and anticancer. Researchers have tested extracts from various parts of the MO tree, both in vitro and in vivo, on several types of cancer (such as liver cancer cells, breast cancer, colorectal, leukemia, lung cancer, and oral cancer) with varying success. This review aims to explore the current state of the latest anticancer activity research of MO plants in the last five years. We tried to explore the anticancer activities of MO extracts from reported in vivo and in vitro studies. We searched systematically from three databases (PubMed, Scopus, and Embase) and summarized the data. The keywords used were "Moringa oleifera" AND "anticancer" AND "in vivo" OR "in vitro". The inclusion criteria were in vivo or in vitro experimental studies and exclusion criteria analyses i.e., in silico trials, study protocols, reviews, or observational studies. This review includes 16 papers on nonclinical studies of MO anticancer activity. Several active compounds have been purified and have reported their anticancer effectiveness, including glucomoringin-ITC/MIC-1, 7-octanoic acid, oleamide, 1-phenyl-2-pentanol, quercetin, gallic acid, p-coumaric acid, and 4-hydroxy 3 – methoxy cinnamic acid, quinic acid. There was no difference in the mechanism of anticancer action based on plant parts, leaves, roots, and seeds, even though using different extraction methods. The general mechanism of action shown was apoptotic, antiproliferative, and cytotoxic. The dose used differed depending on the type of cancer cells used. Some used conventional extraction methods, and others have used modern techniques to extract the purified active compounds from the fractionation process. Our review made it clear that MO could be an excellent and safe candidate for the development of novel therapies against cancer and was most commonly reported in MCF-7, HepG2, and HCT-116 cancer cells. In addition, the development of MO products as future cancer prevention is also interesting to be explored and developed optimally in clinical settings.

Keywords: *Moringa oleifera*, Anticancer, in Vivo, in Vitro.

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1. INTRODUCTION

Cancer is a disease characterized by uncontrolled proliferation of abnormal cells in the body. It has the potential to spread/attack normal cells around it and other parts of the body. Cancer is considered a major cause of morbidity and mortality in many high-income and developing countries (Wu et al., 2021), (Barhoi et al., 2021). Based on the 2018 Riskesdas data, the prevalence of cancer/tumors in Indonesia was found to have increased from 1.4/per 1000 population in 2013 to 1.79/1000 in 2018 (Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan Republik Indonesia, 2019). According to data from the Global Burden of cancer study (Globocon) from the World Health Organization (WHO), the total cancer cases in Indonesia in 2020 reached 396,914 cases and total deaths were 234, 511 cases.

So far, modern cancer treatment that is often done is chemotherapy, radiation therapy, surgery, immunotherapy, and chemotherapy plus adjuvant therapy. However, these interventions result in many side effects, which include nausea, vomiting, fever, pain at the cancer site, causing discomfort to the patient, etc. Treatment with medicinal plants/herbs and new phytochemicals with high effectiveness and low toxicity and side effects are very advantageous (Abd Rani et al., 2019). It is known that 74% of anticancer drugs come from various plant species. MO plant is one of the plants that has been widely reported to have a remarkable effect on the inhibition of various cancer cells (Khor et al., 2018)

All parts of the MO plant have been investigated and proven to have good anticancer activity, including leaves, seeds, bark and roots. Regarding the data we collected, the anticancer research of MO plants both *in vivo* and *in vitro* has mostly focused on leaf extracts. MO leaf extract has been reported to have anticancer activity on liver cancer cells (HepG2)(Barhoi et al., 2021),(Abd-Rabou et al., 2017), breast (MCF-7)(Abd-Rabou et al., 2017),(Mohd Fisall et al., 2021), T47D (Diab et al., 2015), MDA-MB-231(Wisitpongpun et al., 2020), colorectal (HCT-116 (Abd-Rabou et al., 2017),(Diab et al., 2015), Colo-205 (Diab et al., 2015), Caco2 (Abd-Rabou et al., 2017), leukemia (THP-1(Diab et al., 2015), HL-60 (Diab et al., 2015), K562 (Diab et al., 2015), EAC (Barhoi et al., 2021), prostate PC3 (Diab et al., 2015),(Bhadresha et al., 2022), melanoma cells (A375)(Do et al., 2020),(Do et al., 2021), A2058 (Do et al., 2020), (Do et al., 2021), lung cancer (A549)(Diab et al., 2015), cancer cells (HeLa)(Mumtaz et al., 2021). MO seed extract was reported to have anticancer activity on colorectal cells (HCT-116)(Aldakheel et al., 2020), melanoma cells (B16-F10)(de Andrade Luz et al., 2017), liver cancer cells (HepG2) (Elsayed et al., 2015),(Mohamed et al., 2021), breast cancer (MCF-7)(Elsayed et al., 2015), TNB C (Zunica et al., 2021), colorectal (Caco2 and L929) (Elsayed et al., 2015), renal carsinoma cells (RCC), cells 786-O (Xie et al., 2022), OSRC-2(Xie et al., 2022), 769-P (Xie et al., 2022), SK-NEP-1 and ACHN (Xie et al., 2022), and MO root extract on ovarian cancer cells (Ghosh et al., 2021).

Several active compounds/contents of MO which are believed to be responsible for anticancer activity are *4-[(α-L-Rhamnosyloxy) benzyl] isothiocyanate (MIC-1)*19 also called glucomoringin-ITC, MIC-1 can cause inactivation of several transcription factors to inhibit the occurrence and development of inflammation. Another compound is *7-octenoic acid*, *oleamide, 1-phenyl-2-pentanol*(Wisitpongpun et al., 2020), *quercetin* (Mohamed et al., 2021), *gallic acid, p-coumaric acid*, and *4-hydroxy 3- methoxy cinnamic acid* (Mumtaz et al., 2021), *quinic acid* (Barhoi et al., 2021). The compound was purified from the MO extract and was tested singly.

The mechanism of action of MO plants as anticancer is reported to slow the cancer process through targeting chemoprevention, inhibiting carcinogenic activation, inducing carcinogen detoxification, anti-inflammatory, anti-tumor cell proliferation, activating hormones and enzymes, stimulating DNA repair mechanisms, and increasing the production of protective enzymes that generate antioxidants and induce cancer cell apoptosis. At the same time, it is also proven that MO has excellent potential to inhibit the development of

cancer/tumor without affecting the normal physiology and function of the body; hence, it can be used as a cancer treatment drug.

According to our knowledge, what is exciting and not yet known is whether different parts of MO plants (leaves, roots, bark, seeds, flowers), extraction methods, solvent, and doses used will show differences in anticancer mechanism and potential for success. This review aims to explore various studies on the anticancer activity of MO in the last five years, both in vivo and in vitro. The study focused on plant parts, extracts, extraction methods, solvents, in vivo/in vitro test methods, types of cancer cells, the doses used, the IC50 value, and the effects. The review results are anticipated to provide critical information for developing the synthesis of active MO compounds, which could lead to new cancer drugs. To date, no clinical trials involving MO extract for cancer treatment in humans have been reported.

2. RESEARCH METHOD

We used several electronic databases: PubMed, Scopus, and Embase, from May 1 to June 12, 2021. The keywords used in the search strategy were:

Pubmed: TITLE/ABS (*Moringa oleifera*) AND (anticancer) OR (in vivo) OR (in vitro)

Scopus : TITLE-ABS-KEY (*Moringa oleifera*) AND TITLE-ABS-KEY (anticancer) AND TITLE-ABS-KEY (in-vivo) AND TITLE-ABS-KEY (in vitro)

Embase: TITLE-ABS-KEY (*Moringa oleifera*) AND (anticancer) OR (in vivo)) OR (in vitro)

The studies assessed were in vitro/in vivo/animal studies on MO as an anticancer agent and were limited to the last five years. We included randomized or non-randomized controlled trials and in vivo or in vitro experimental studies. Articles with irrelevant study designs were excluded from analyses i.e., in silico trials, study protocols, reviews, or observational studies. The first author (NTK) extracted data from an electronic database that met the eligibility criteria.

The extracted data were then examined by a second author (EHP). We reported non-clinical data (in vitro and in vivo), namely the author's name and year of publication, MO extract used, MO dose and type of control, duration, cancer cell type, and effects for nonclinical trials. Safety results were reported when available. The three reviewers (NTK, EHP, and ML) checked the extracted data, followed by discussion by all reviewers to harmonize perceptions and resolve any differences.

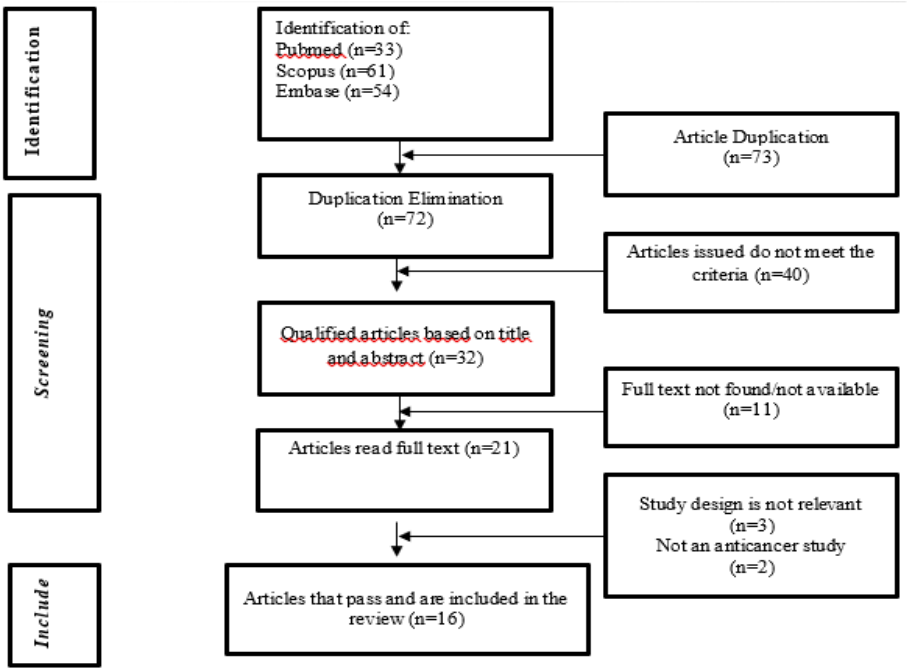

Figure 1. Flow of article selection in research

3. RESULTS AND DISCUSSION

Table 1. Non Clinical Study of *Moringa oleifera* Lam as Anticancer.

Author, year (Reference)	**Type of extracts/main bioactive compound used**	**Methods of extracts/ bioactive preparation**	**In vitro/in vivo models used**	**Treatment dosis/ duration**	**Toxicity /IC50**	**Effects**	**Mechanism**
Abd Rabou, 2017 (Abd-Rabou et al., 2017)	Nano MO leaves extracts, root core (Rc) and outer (Ro)	Nanoparticle Manufacturing PLGA-CS-PEG	**In vitro** MTT Assay using hepatocarcinoma HepG2, breast MCF7, and colorectal HCT 116/Caco-2 cells and normal kidney BHK-21 cell lines	(0, 20, 40, 60, 80, 100 µM) at 37°C for 4 h.	- Caco2 (Rc=32,16µM), (Ro=39,51 µM) - HCT-116 (Rc=29,14 µM), (Ro=51,42 µM) - HepG2 (Rc=29,1 µM), (Ro=53,9 µM) - MCF7 (Rc=46,15 µM), (Ro=>100) - BHK-21 (Rc=>100 µM), (Ro=49,75 µM)	- inhibited cell proliferation and exhibited apoptosis-mediated cell death in HepG2, HCT 116 and Caco-2, MCF7, - Rc extract is safe on BHK-21 cells with minimal cytotoxic effect	Apoptosis

Aldakheel RK, 2020 (Aldakheel et al., 2020)	MO seeds etanol extracts	Diversified ratios of the MO powder between 5–25 g was mingled with 220 mL of ethanol and were stirred for 5 h.	**In vitro** MTT Assay using colorectal cells HCT-116 and normal cells (HEK-293)	(30–100 µg/mL) for 4 h	Not mention	- Inhibition of HCT-116 . cell growth - Does not inhibit HEK-293 . cells	inhibit cell proliferation
Barhoi, 2021(Barhoi et al., 2021)	MO leaves aquaeos extracts	Soxhletation Method (30 g with 200 mL water for 6-8 hours	**In vivo** Anti-tumor activity of AEMO was assessed in EAC (Ehrlich acites carcinoma) induced solid tumor bearing mice by analyzing tumor weight (TW) and Tumor volume (TV). **In vitro** MTT Asay and trypan blue using EAC and Hep-2 cells	**In vivo** 200 and 400 mg/kg BW of MO aquaeos extracts **In vitro** (0.05, 0.1, 0.25, 0.5 (1mg/ml) for both 48h and 72h	Not mention	- Reduce tumor size - Maintain levels of AST, ALT, urea, Hb, WBC, RBC, - cytotoxic to cancer cells and reduces the potential for tumor cell proliferation, thereby increasing the survival time of mice containing tumors.	- Apoptosis alters the mitochondrial membrane potential - antiproliferation

Bhadresha, 2022(Bhadresha et al., 2022)	MO leaves aquaeos extracts	10 grams of dried leaf powder were boiled with 100 mL of distilled water (70 °C) with stirring for 30-60 minutes on a hot plate, centrifuged at 1,000 g for 10 minutes at room temperature. The collected top aqueous layer was stored at 40 °C.	***In vitro*** human prostate bone metastasis cell line (PC3)	100–400 µg/mL for 24 hr	Not mention	- inhibition PC3 cell growth - increase Bax gene expression and decrease Bcl2 expression	Apoptosis
de Andrade, 2017(de Andrade Luz et al., 2017)	MO seeds lectin	Preparation of coagulant M. oleifera lectin (cMoL), MO seed powder was	**In vitro** Trypan blue assay and analisis flowcytometry B16-F10 murine melanoma cells	cMoL (1.5–16 µM) reduced	9.72 µM	- induces necrosis and suggests the presence of cells in late apoptosis.	apoptosis

		extracted with 0.15 M NaCl at 25 °C for 6 h.				- The specificity for tumor cells was observed since the death of normal human fibroblasts (GN) was not higher than 20% in the treatment with cMoL from 1.5 to 16 M - cMoL increased mitochondrial ROS production and promoted caspases 3, 8 and 9 activation in B16-F10 cells, indicating the activation of apoptosis-related pathway	
Diab, K.A, 2015(Diab et al., 2015)	MO leaves etanol 50% extract	Percolation Methods	**In vitro** SRB and MTT	(10, 30, 50, 70 and 100 µg/mL)	(µg/mL) - A549 13.2±1.8	Inhibits cell growth	cytotoxic and antiproliferation

			Assay on cancers of lung (A549), prostate (PC-3), breast (T47D and MCF-7), colon (HCT-16 and Colo-205) and leukemia (THP-1, HL-60 and K562)		- PC-3 (22.2±4.9) - T74D (33.5±2.5) - MCF-7 (26.4±5.7) - HCT-16 (28.8±2.2) - Colo-205 (49.7±0.8) - THP-1 (35.8±1.7) - HL-60 (50.0±1.0) - K562 (149.9±1.7)		
Do.B.H, 2020(Do et al., 2020)	MO leaves aquaeos extracts	Dried MO leaf powder (200 g) was incubated with boiling water (95°C, 7,500 mL) for 2 hours and then vacuumed.	**In vitro** WST-1 assay on Melanoma cells A375 and A2058 cells	(0–200 µg/mL) for 24–72 h	Not mention	- increased Bax/Bcl-2 ratio, leading to loss of mitochondrial membrane integrity - activation of Caspase 3/7, Caspase 9, PARP cleavage and Caspase independent pathways via	inhibits cell proliferation and induces apoptosis in human melanoma cells A375

						AIF translocation	
Do.B.H, 2021(Do et al., 2021)	MO leaves aquaeos extracts	Ultrasonic assisted extraction (40 kHz) with methanol for 3 hours. Fractination	**In vitro** WST-1 assay on Melanoma cells A375 cells and A2058 cells. Apoptotic activity was assessed by DNA condensation, DNA fragmentation, and phosphatidylserine (PS) externalization assays.	(25, 50, 75 µg/ml) for 48 h	- A375 cells (40.80 ± 2.12 µg/ml) - A2058 cells. (52.74 ± 1.87 µg/ml)	- increased ROS production and reduced mitochondrial membrane potential. - MO activates Bax while reducing Bcl-2 expression, leading to an increase in the Bax/Bcl-2 ratio. - activation of Caspase-3/7 and Caspase-9 (Caspase-dependent pathway), activation and translocation of apoptosis-inducing factor (AIF) into the nucleus (Caspase-independent pathway).	DNA condensation and DNA fragmentation

Elsayed, 2015(Elsayed et al., 2015)	MO seeds aquaeos extracts	Oil extracted by cold pressing	**In vitro** MTT assay on HeLa, HepG2, MCF-7, Caco-2 and L929 cell lines	15.6 µg/mL to 1 mg/mL.	- MCF-7 cells (226.1 µg/mL), - HeLa cells (422.8 µg/mL) - HepG2 cells (751.9 µg/mL)	significant decrease in cell viability	cytotoxic activity
Ghost.A, 2021(Ghosh et al., 2021)	MO root aquaeos extracts	MO root powder extracted in water filtered with sterile gauze, 0.45 mm porous. OAW42 paper.	**In vitro** MTT assay, clonogenic assay, cell cycle analysis, flow cytometry, western blot analysis, immunocytochemical analysis of FSHRand c-Myc expression. **In vivo** tumour xenograft mouse model.	Not Mention	(Cisplatin: dose used in combination 0.25 mM, IC-50 value: 0.35 mM; Paclitaxel: dose used in combination 0.035 nM, IC-50 value: 0.058 nM) along	- MO root extract exhibited cytotoxic activity, induces apoptosis, and attenuated expression of FSHR and c-Myc in OAW42 ovarian cancer cells. - MRE attenuates the development of a mouse model of ovarian carcinoma through	antiproliferative effect and antagonistic role of FSHR

						downregulation of FSHR, c-Myc and PECAM1. - The MO extract also attenuated the expression of CD31, FSHR, and c-Myc in a tumour xenograft mouse model	
Mohamed. M, 2021(Mohamed et al., 2021)	MO leaves and seeds methanol extracts.	MO leaf powder 100g was macerated with 80% methanol. MO seed powder is extracted by 3 methods (cold press, solvent extraction, and ultrasound-assisted extraction).	**In vitro** Screening of hepatoprotective activity on the protection of human liver-derived HepG2 cells against CCl4-induced damage. MTT Assay on HepG2 . cells	0, 20, 40, 60, 80, and 100 µg/ml	- Petroleum ether fraction(19.25 µg/ml, - methanolic extract 21.09 µg/ml, - methylene chloride fraction21.52 µg/ml.	- hepatoprotective effect - Inhibits the growth of cancer cells	cytotoxic activity

Jing Xie, 2021(Xie et al., 2022)	MO seeds petroleum eter extracts.	Soxhlet extractor	**In vitro** renal cell carcinoma (RCC) cells 786-O, OSRC-2, 769-P, SK-NEP-1, and ACHN **In vivo** 6-week-old male BALB/C nude mice. After 1 week of adaptive feeding, 786-O cells (5×10^6) were subcutaneously injected into each mouse.	**In vitro** (0, 1, 2, and 4 μM) for 24 h or 48 h. After 24 or 48 h **In vivo** MIC-1 low-dose (25 mg/kg) group, and a MIC-1 high-dose (50 mg/kg) group	Not mention	induce apoptosis and cell cycle arrest, increase the Bax/Bcl-2 ratio, and decrease expression of cell cycle-associated proteins in 786-O cells and 769-P cells.	inhibits PTP1B-mediated activation of the Src/Ras/Raf/ERK signaling pathway.
Umiey F. M. Fisall, 2021(Mohd Fisall et al., 2021)	MO leaves Methanol extracts.	Maceration, with methanol 80%	**In vitro** breast cancer cells (MCF7).		5 μg/mL	increased expression of the pro-apoptotic proteins Bax, caspase 8 and p53 in MCF7 . cells	inhibit the proliferation of MCF7 cells through the induction of apoptosis
Mumtaz, 2021(Mumtaz et al., 2021)	MO leaves Methanol extracts. (Fraksinatio n)	Percolation with Methanol 80%	**In vitro** MTT Assay on HeLa cells	26, 52, 104, 206, and 416 μg/mL	Not mention	- chloroform fraction (CF) followed by n-hexane (HF) and butanol	- increased apoptosis through downregulation of E2F1 and

						(BF) fraction showed the highest anticancer activity - While the water fraction (AF) followed by the ethyl acetate (EAF) fraction showed lowest anticancer activity - decreased cell viability in HeLa cancer cells	upregulation of Bax protein. - cessation of cancer survival pathways including the NF-Kβ signaling cascade via downregulation of p65 components.
Wisitpongpun. P, 2020 (Wisitpongpun et al., 2020)	MO leaves hexane, ethyl acetate (EtOAc) extracts (fraksination)	Not mention	**In vitro** MTT Assay on Breast Cancer Cells MDA-MB-231	75, 100, and 150 µg/mL for 24 h.	- Etyl asetat EtOAc extracts (233.5 µg/mL) - Etanol EtOH extracts (241.1 µg/mL), - hexane extract (342.6 µg/mL)	significant reduction in cell viability, striking reduction in colony formation, and induction of apoptosis and cell cycle arrest in the G2/M phase.	stop the cell cycle and induce apoptosis of MDA-MB-231 cells
Zunica E.R.M, 2021 (Zunica et al., 2021)	MO seeds extracts	The seed powder was incubated in	**In vitro**	**In vivo** MO concentrate (MC; HFD	Not Mention	- Single MO supplementation does not	reduce angio-genesis in tumors

	water at a controlled temperature with constant stirring.	Triple negative breast cancer (TNBC) **In vivo** female mice at 4 weeks of age induced tumor xenograft derived from MDA-MB-231- and obesity	supplemented with 0.6% w/w MC), MO plus chemotherapy (MC + Chemo; HFD supplemented with 0.6% w/w MC with weekly 2 mg/kg doxorubicin and 100 mg/kg cyclophosphamide IP injections)	attenuate tumor growth, in combination with chemotherapy worsens tumor development. - Single MO supplementation reduces angiogenesis, but this effect is abrogated in combination with chemotherapy.

Most of the researchers in this study started by examining the antioxidant and anti-inflammatory activity of MO leaf extract as an initial screening for anticancer activity. It is known that oxidative stress occurs when there is an imbalance between the production of free radicals and antioxidants. Antioxidants can decrease free radical formation, reduce oxidative stress, and potentially prevent cancer.

An in vitro assay will assess the impact of MO extract on cancer cell growth, proliferation, and morphology. If the results demonstrate significant anticancer activity without harming normal cells, molecular analysis will be conducted to identify the specific pathway disrupted by the extract. Subsequently, in vivo studies will be conducted to assess the anticancer efficacy of MO extracts using rat or mouse models with induced cancer.

The reviewed articles demonstrated antioxidant and anticancer properties of MO extracts in both in vitro and in vivo models, as well as elucidating the underlying pathways and mechanisms of action.

Types of Plants Parts and Extraction Method

Our search results for the last five years (2017-2022) show that the most widely used parts of the MO plant in anticancer research are leaves, 12/16 articles (67%), 5 articles using seeds and 2 articles using roots. MO leaves are rich in polyphenols and polyflavonoids, which are potent antioxidants and anticancer compounds. The solvent used also varies, polar: water (7) > *ethanol* (4) > *methanol* (3); and non polar *n-hexane* (1)> *ethyl acetate*(1) > *petroleum ether* (1).

Several extraction methods used in the reviewed studies aim to be able to attract as many bioactive components as possible. Conventional extraction methods are still used, including stirring using magnetic stirrer (Aldakheel et al., 2020), soxhletation (Barhoi et al., 2021)·(Xie et al., 2022), soxhletation for oil extraction (Das et al., 2024), boiling with hot water (Bhadresha et al., 2022)·(Do et al., 2021), percolation(Diab et al., 2015), and maseration (Mohd Fisall et al., 2021)·(Mohamed et al., 2021). Non-conventional/modern methods also continue to develop in updated research, including the manufacture of nanoparticles (Abd-Rabou et al., 2017), manufacture of coagulant MO *lectin* (cMoL)(de Andrade Luz et al., 2017), ultrasonic assisted extraction (UAE)(Do et al., 2021), ultrasound and microwave (Esparza et al., 2024), cold pressing(Mohamed et al., 2021)·(Zunica et al., 2021) notable for MO seeds.

The analysis found that the distribution of bioactive/phytochemical components varied in the MO plant parts (leaves, seeds, roots) and that different extraction processing techniques could affect the withdrawal of the active substances; thus, it is necessary to choose a well-characterized extraction method.

Compounds believed to act as anticancer

One of the compounds that has been tested for its effectiveness as an anticancer is 4-*[(α-L-Rhamnosyloxy) benzyl] isothiocyanate* (MIC-1). Recent research from Xie Jing, et al proved that MIC-1 isolated from MO seeds with a concentration of 10 M significantly inhibited the growth of 5 RCC cell lines including cells 786-O, OSRC-2, 769-P, SK-NEP-1, and ACHN, but not toxic to normal renal cells (HK2). It also showed that MIC-1 suppressed the migration and invasion ability of 786-O and 769-P cells, and reduced expression of matrix metalloproteinases (MMP)-2 and MMP-9. Furthermore, MIC-1 induced apoptosis and cell cycle arrest increased the Bax/Bcl-2 ratio and decreased expression of cell cycle-associated proteins in 786-O cells and 769-P cells. (Xie et al., 2022)

The investigated mechanism of action is that MIC-1 can suppress 786-O cell growth and migration by inhibiting the PTP1B-mediated activation of the Src/Ras/Raf/ERK signalling pathway. Further, *in vivo* experiments demonstrated that MIC-1 inhibited xenograft tumor growth in mice, and increased the Bax/Bcl-2 ratio in tumor tissue. Moreover, MIC-1 had no

effect on the PTP1B-dependent Src/Ras/Raf/ERK signalling pathway in HCT-116 cells, Hep-G2 cells, and cell A431.(Xie et al., 2022)

Zonica, et al also reported that MIC-1 assay as a single supplementation did not attenuate tumor growth but, in combination with chemotherapy, decreased tumor progression. In addition, single MO supplementation reduced angiogenesis, but this effect was abolished in combination with chemotherapy.(Zunica et al., 2021)

Rajan, et al has previously reported *in vitro* antitumor activity of glycosylated moringin isothiocyanate [*4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate*] resulting from quantitative *myrosinase-induced hydrolysis* of glucomoringin (GMG) under neutral pH values. Its glucosinolate precursor isothiocyanates (ITCs) have been shown to inhibit tumorigenesis of Astrocytoma grade IV, the most common and most malignant primary brain tumour in adults.(Rajan et al., 2016)

Rajan et al also showed the potency of moringin to induce apoptosis and cell death of human astrocytoma CCF-STTG1 grade IV. Moringin was shown to induce apoptosis through p53 and Bax activation and Bcl-2 inhibition. In addition, oxidative stress-associated transcription factor Nrf2 and upstream regulator of CK2 alpha expression at higher doses suggest the involvement of moringa-induced oxidative stress-mediated apoptosis.(Rajan et al., 2016)

While the extraction of bioactive components often requires more than a single method, recent research has advanced the field by employing fractionation with different solvents to pinpoint specific bioactive compounds responsible for observed effects. This approach, as reported by Wisitpongpun et al. (2020), Muntaz et al. (2021), and Mohamed et al. (2021), has proven effective in identifying these crucial components. Wisitpongpun, et al reported that MO leaves were sequentially extracted with hexane, ethyl acetate (EtOAc), and ethanol. MO extracts and their derivative fractions were continuously screened for anticancer activity. They selected the most decisive bit for refraction and identified ten bioactive compounds using LC-ESI-QTOF-MS/MS analysis. The EtOAc extracts and their fractions were screened for anticancer activity using different bioassays. The study concluded that oleamide has the strongest potential as an anticancer treatment by inducing apoptosis through suppression of Bcl-2 expression and further promoting caspase three activation. This is one of the first report regarding MOL extract and oleamide activity against the MDA-MB-231 cell line.(Wisitpongpun et al., 2020)

Muntaz, et al reported that the chloroform fraction (CF) followed by n-hexane (HF) and butanol (BF) fractions showed the highest anticancer activity while the water fraction (AF) followed by ethyl acetate (EAF) fraction showed the lowest anticancer activity and decreased cell viability in cancer cells (HeLa). In vitro and in silico results showed that *quercetin, gallic acid, p-coumaric acid*, and *4-hydroxy 3-methoxycinnamic acid* showed strong anticancer activity and good therapeutic potential against cancer. (Mumtaz et al., 2021)

Mohamed. M, et al also reported that different fatty acids were identified in MO seed oil with solid biological activities such as antioxidant activity, demonstrated by palmitic and oleic acids. Futher, the hepatoprotective activity for palmitoleic acid was reported. Additionally, oleic acid demonstrated potent anticancer activity. These findings underscore the biological observation that plant seed oil exhibits both hepatoprotective and cytotoxic properties.So, further biological studies should be carried out to make optimal use of the obtained MO seed oil, especially by extraction using ultrasonic technique. (Mohamed et al., 2021)

Types of Cancer Cells

Our study showed that the most common types of cancer cells used for anticancer activity of MO leaves and MO seeds were HepG2, HCT-116, and Caco2. Most of the articles focused

on the leaves. Several types of cancer cells that have been tested are liver cancer cells (HepG2) (Barhoi et al., 2021),(Abd-Rabou et al., 2017), breast (MCF-7)(Abd-Rabou et al., 2017),(Mohd Fisall et al., 2021), T47D(Diab et al., 2015), MDA-MB-231(Wisitpongpun et al., 2020)) colorectal (HCT-116)(Abd-Rabou et al., 2017),(Diab et al., 2015), Colo-205(Diab et al., 2015), Caco2(Abd-Rabou et al., 2017)), leukemia (THP-1(Diab et al., 2015), HL-60(Diab et al., 2015), K562(Diab et al., 2015), EAC(Barhoi et al., 2021)), prostate PC3(Diab et al., 2015),(Bhadresha et al., 2022), melanoma cell (A375(Do et al., 2020),(Do et al., 2021), A2058(Do et al., 2020),(Do et al., 2021)), lung cancer (A549)(Diab et al., 2015), , cancer cell HeLa(Mumtaz et al., 2021). MO Seed Extract was reported to have anticancer activity on HCT-116. colorectal cells(Aldakheel et al., 2020), melanoma cell B16-F10(de Andrade Luz et al., 2017), liver cancer cells HepG2(Elsayed et al., 2015),(Mohamed et al., 2021), breast cancer (MCF-7(Elsayed et al., 2015), TNBC(Zunica et al., 2021)), colorectal Caco2 and L929(Elsayed et al., 2015)), renal cell carcinoma (RCC), cell 786-O(Xie et al., 2022), OSRC-2(Xie et al., 2022), 769-P(Xie et al., 2022), SK-NEP-1 and ACHN(Xie et al., 2022), CAL27 and SCC15 (Das et al., 2024) and root extract on ovarian cancer cells.(Ghosh et al., 2021) It was not explained in every article the basis for selecting the type of cancer cell.

Mechanism of action in vitro and in vivo

Apoptosis is programmed cell death. It comes from the Greek word, which means fall (falling off). Apoptotic cells experience morphological changes such as cell shrinkage, chromatin condensation, and nuclear fragmentation.(Luo et al., 2020) In general, apoptosis is divided into three phases, namely the induction phase, the effector phase, and the degradation phase. The induction phase depends on signals that cause cell death including reactive oxygen species (ROS), B-cell lymphoma-2 (Bcl-2) family proteins such as Bcl-2 associated x protein (Bax) and Bcl-2 associated death promoter (Bad). During the effector phase, the cell undergoes death due to the actions of the regulatory center, the mitochondria.The last phase, namely the degradation phase, involves a series of events that occur both in the cytoplasm and in the cell nucleus.(Edlich, 2018)

The regulation of apoptosis is largely determined by the ratio between Bcl-2 and Bax to determine whether the cell will react by becoming apoptotic or surviving. There are several models to describe the ratio of prosurvival (Bcl-2) and proapoptosis (Bax) in inhibiting or causing apoptosis (Figure 2). The first model shows that Bcl-2 inhibits apoptosis, and Bax removes this inhibition resulting in apoptosis. The second model states that Bax induces apoptosis, and Bcl-2 inhibits this induction. The third model is a model that mentions the existence of interdependence. Bcl-2 inhibits apoptosis and Bax induces it.(Hadi, 2011),(Hafezi & Rahmani, 2021) However, apoptosis is actually a combination of the three existing models and occurs complexly.

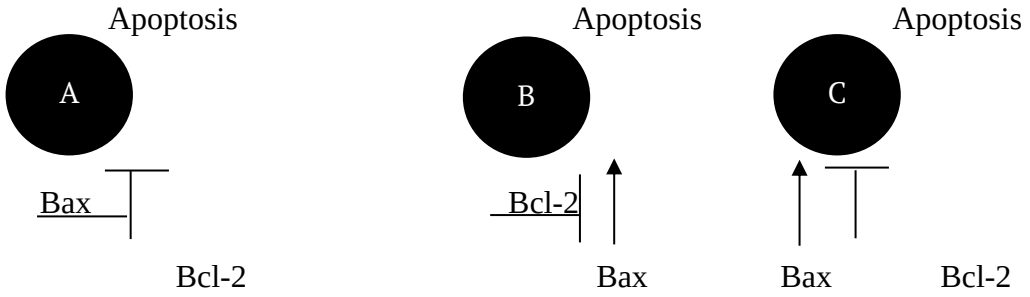

Figure 2. Model of the ratio of Bcl-2 and Bax in causing apoptosis (Chao and Korsmeyev, 1998 in Hadi, 2011).

Based on our study, most of the mechanisms of action are apoptosis and antiproliferation. Extracts derived from plant parts mostly showed the mechanism of action of inhibiting cancer

cell growth and increasing Bax gene expression, and decreasing Bcl-2 expression.(Bhadresha et al., 2022) The coagulant MO lectin cMoL derived from seeds showed cMoL activity increased mitochondrial ROS production and promoted activation of caspases 3, 8 and 9 in B16-F10 cells, suggesting activation of apoptosis-associated pathways.(de Andrade Luz et al., 2017) Do.B.H, et al used the same plant parts, namely MO leaves with different extraction methods but found the same mechanism of action, namely an increase in the Bax/Bcl-2 ratio, which causes loss of mitochondrial membrane integrity, activation of Caspase 3/7, Caspase 9, cleavage PARP and Caspase-independent pathways via AIF translocation.(Do et al., 2020)·(Do et al., 2021)

MO root extract reported by Ghost et al showed slightly different mechanisms, accentuated antiproliferative/cytotoxic effects, and antagonistic role of FSHR wherein MO root extract exhibited cytotoxic activity, induces apoptosis, and attenuated expression of FSHR and c-Myc in ovarian cancer cells OAW42.(Ghosh et al., 2021) In vivo studies using animal models reported that the MRE mechanism attenuated the development of mouse ovarian carcinoma models through downregulation of FSHR, c-Myc and PECAM1. The MO extract also attenuated the expression of CD31, FSHR, and c-Myc in a mouse model of xenograft tumors.

Recent research by Susanto et al, on MO powder in the form of bio-fabricated silver nanoparticles (AgNPs) showed effectiveness in reducing the viability and proliferation of HT-29 cells. In addition, MO AgNPs also reduced the expression of genes associated with metastasis. These findings suggest that MO AgNPs may be considered as a potential strategy for the treatment of colon cancer. (Susanto et al., 2024). To fully utilize the potential of Moringa nanoparticles, future research should focus on increasing production, optimizing the synthesis process, and investigating alternative applications. (Choudhary et al., 2024). Standardized procedures for quality control and quantification of bioactive compounds are essential to ensure consistent and effective application in the pharmaceutical industry. In vivo toxicity, safety tests and even clinical trials are essential, especially for long-term use. (Das et al., 2024).

We recommend the development of active compounds of MO as industrially developed anticancer candidates. Clinical trials of MO pharmaceutical products that have been formulated and believed to be stable and effective are necessary for the future progress of the pharmaceutical industry. In-depth research at the cellular and molecular level is essential to continue discovering potential active compounds. Development of the most effective clinical trial design is needed to test active compounds that have been proven in vitro to inhibit cancer cell growth. Utilization of MO formula preparations as a companion adjuvant to synthetic cancer drugs is also needed for further research.

The use of MO preparations as traditional medicine, such as brews, herbs, extracts, powders, flour, gels, and creams, has drawbacks including non-specific therapeutic targets and potential side effects from unintended active ingredients. Consequently, MO-based traditional therapies are unlikely to be effective for treating advanced-stage cancer.Purification of specific compounds from traditional MO preparations is necessary to obtain effective and maximum therapeutic effects. Clinical trials to validate therapeutic claims and studies on interactions with conventional drugs are essential for evidence-based use of MO.

Several limitations were encountered in this study. The majority of references were derived from in vitro studies, with a paucity of in vivo data. Furthermore, there was a dearth of reports on in vivo efficacy or clinical trials investigating the anticancer properties of MO. The search was restricted to three databases (Scopus, Embase, PubMed), which may have contributed to the limited number of retrieved references. Additionally, we were unable to identify and report on each specific active compound responsible for the observed anticancer activity.

4. CONCLUSION

We did not find any difference in the mechanism of action of the leaves, roots, and seeds even at different extraction methods. The general mechanism of action shown is apoptotic, antiproliferative, and cytotoxic. The doses used differed depending on the type of cancer cells used. Some articles used extracts, but others have used active compounds purified from the fractionation process. Thus, we demonstrate that MO plants could be excellent and safe candidates for the development of new therapies against cancer.

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REFERENCES

Abd Rani, N. Z., Kumolosasi, E., Jasamai, M., Jamal, J. A., Lam, K. W., & Husain, K. (2019). In vitro anti-allergic activity of Moringa oleifera Lam. extracts and their isolated compounds. *BMC Complementary and Alternative Medicine*, *19*(1), 1–16. https://doi.org/10.1186/s12906-019-2776-1

Abd-Rabou, A. A., Abdalla, A. M., Ali, N. A., & Zoheir, K. M. A. (2017). Moringa oleifera root induces cancer apoptosis more effectively than leave nanocomposites and its free counterpart. *Asian Pacific Journal of Cancer Prevention*, *18*(8), 2141–2149. https://doi.org/10.22034/APJCP.2017.18.8.2141

Aldakheel, R. K., Rehman, S., Almessiere, M. A., Khan, F. A., Gondal, M. A., Mostafa, A., & Baykal, A. (2020). Bactericidal and in vitro cytotoxicity of moringa oleifera seed extract and its elemental analysis using laser-induced breakdown spectroscopy. *Pharmaceuticals*, *13*(8), 1–18. https://doi.org/10.3390/ph13080193

Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan Republik Indonesia. (2019). *Laporan Nasional Riskesdas 2018*. Jakarta: Badan Penelitian dan Pengembangan Kesehatan, Kementerian Kesehatan Republik Indonesia.

Barhoi, D., Upadhaya, P., Barbhuiya, S. N., Giri, A., & Giri, S. (2021). 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*, *40*(1), 70–85. https://doi.org/10.1080/07315724.2020.1735572

Bhadresha, K. P., Jain, N. K., & Rawal, R. M. (2022). Assessing the protective effect of Moringa oleifera extract against bone metastasis: an invitro simulated digestion approach. *Nutrition and Cancer*, *74*(3), 1023–1036. https://doi.org/10.1080/01635581.2021.1933099

Choudhary, R., Kumari, A., Kachhwaha, S., Kothari, S. L., & Jain, R. (2024). Moringa oleifera: Biosynthesis strategies for enhanced metabolites and role in green nanoparticle synthesis. In *South African Journal of Botany* (Vol. 170, pp. 271–287). Elsevier B.V. https://doi.org/10.1016/j.sajb.2024.05.009

Das, M., Mohanty, J. N., Bhuyan, S. K., & Bhuyan, R. (2024). Anti-cancer activity of Moringa oleifera Lam. seed oil on oral cancer. *Beni-Suef University Journal of Basic and Applied Sciences*, *13*(1). https://doi.org/10.1186/s43088-024-00475-z

de Andrade Luz, L., Rossato, F. A., Costa, R. A. P. e., Napoleão, T. H., Paiva, P. M. G., & Coelho, L. C. B. B. (2017). Cytotoxicity of the coagulant Moringa oleifera lectin (cMoL) to B16-F10 melanoma cells. *Toxicology in Vitro*, *44*(February), 94–99. https://doi.org/10.1016/j.tiv.2017.06.019

Diab, K. A. E., Guru, S. K., Bhushan, S., & Saxena, A. K. (2015). In vitro anticancer activities of Anogeissus latifolia, Terminalia bellerica, Acacia catechu and Moringa oleiferna Indian plants. *Asian Pacific Journal of Cancer Prevention*, *16*(15), 6423–6428. https://doi.org/10.7314/APJCP.2015.16.15.6423

Do, B. H., Hoang, N. S., Nguyen, T. P. T., Ho, N. Q. C., Le, T. L., & Doan, C. C. (2021). 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*, *73*(5), 869–888. https://doi.org/10.1080/01635581.2020.1776885

Do, B. H., Nguyen, T. P. T., Ho, N. Q. C., Le, T. L., Hoang, N. S., & Doan, C. C. (2020). Mitochondria-mediated Caspase-dependent and Caspase-independent apoptosis induced by aqueous extract from Moringa oleifera leaves in human melanoma cells. *Molecular Biology Reports*, *47*(5), 3675–3689. https://doi.org/10.1007/s11033-020-05462-y

Edlich, F. (2018). BCL-2 proteins and apoptosis: Recent insights and unknowns. *Biochemical and Biophysical Research Communications*, *500*(1), 26–34. https://doi.org/10.1016/j.bbrc.2017.06.190

Elsayed, E. A., Sharaf-Eldin, M. A., & Wadaan, M. (2015). 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*, *16*(11), 4671–4675. https://doi.org/10.7314/APJCP.2015.16.11.4671

Esparza, C., Estrada, R., Sanchez, D. S., Saenz-Galindo, A., Valdes, J. A. A., Flores-Gallegos, A. C., & Rodríguez-Herrera, R. (2024). Ultrasound and microwave extraction from Moringa oleifera Lam.: Characterization and antiproliferative effect. *International Journal of Secondary Metabolite*, *11*(2), 292–304. https://doi.org/10.21448/ijsm.1363300

Ghosh, A., Roychowdhury, T., Nandi, R., Maiti, R., Ghosh, N. N., Molla, S. A., Mukhopadhyay, S., Prodhan, C., Chaudhury, K., Das, P., Sarkar, N. K., Chattopadhyay, S., Bhattacharya, R., Bose, C. K., & Maiti, D. K. (2021). Inhibitory role of a smart nano-trifattyglyceride of Moringa oleifera root in epithelial ovarian cancer, through attenuation of FSHR - c-Myc axis. *Journal of Traditional and Complementary Medicine*, *11*(6), 481–492. https://doi.org/10.1016/j.jtcme.2021.03.005

Hadi, R. S. (2011). Mekanisme apoptosis pada regresi sel luteal. *Majalah Kesehatan PharmaMedika*, *3*(1), 246–254.

Hafezi, S., & Rahmani, M. (2021). Targeting BCL-2 in Cancer: Advances, Challenges, and Perspectives. *Cancers*, *13*(1292), 1–15.

Khor, K. Z., Lim, V., Moses, E. J., & Abdul Samad, N. (2018). The in vitro and in vivo anticancer properties of Moringa oleifera. *Evidence-Based Complementary and Alternative Medicine*, *2018*. https://doi.org/10.1155/2018/1071243

Luo, X., O'Neill, K. L., & Huang, K. (2020). The third model of Bax/Bak activation: A Bcl-2 family feud finally resolved? *F1000Research*, *9*, 1–15. https://doi.org/10.12688/f1000research.25607.1

Mohamed, M., Ibrahim, M., Abdel-Azim, N., & El-Missiry, M. (2021). Chemical and biological studies on Moringa oleifera L. cultivated in Egypt. *Egyptian Pharmaceutical Journal(Egypt)*, *20*(1), 33–41. https://doi.org/10.4103/epj.epj_37_20

Mohd Fisall, U. F., Ismail, N. Z., Adebayo, I. A., & Arsad, H. (2021). 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*, *48*(5), 4465–4475. https://doi.org/10.1007/s11033-021-06466-y

Mumtaz, M. Z., Kausar, F., Hassan, M., Javaid, S., & Malik, A. (2021). Anticancer activities of phenolic compounds from Moringa oleifera leaves: in vitro and in silico mechanistic study. *Beni-Suef University Journal of Basic and Applied Sciences*, *10*(1). https://doi.org/10.1186/s43088-021-00101-2

Rajan, T. S., De Nicola, G. R., Iori, R., Rollin, P., Bramanti, P., & Mazzon, E. (2016). Anticancer activity of glucomoringin isothiocyanate in human malignant astrocytoma cells. *Fitoterapia*, *110*, 1–7. https://doi.org/10.1016/j.fitote.2016.02.007

Susanto, H., Firdaus, S. R. A., Sholeh, M., Endharti, A. T., Taufiq, A., Malek, N. A. N. N., & Permatasari, H. K. (2024). Moringa oleifera Leaf Powder – Silver Nanoparticles (MOLP-AgNPs) efficiently inhibit metastasis and proliferative signaling in HT-29 human colorectal cancer cells. *Journal of Agriculture and Food Research*, *16*. https://doi.org/10.1016/j.jafr.2024.101149

Wisitpongpun, P., Suphrom, N., Potup, P., Nuengchamnong, N., Calder, P. C., & Usuwanthim, K. (2020). In vitro bioassay-guided identification of anticancer properties from Moringa oleifera lam. Leaf against the mda-mb-231 cell line. *Pharmaceuticals*, *13*(12), 1–20. https://doi.org/10.3390/ph13120464

Wu, Y. Y., Xu, Y. M., & Lau, A. T. Y. (2021). Anti-cancer and medicinal potentials of moringa isothiocyanate. *Molecules*, *26*(24). https://doi.org/10.3390/molecules26247512

Xie, J., Qian, Y. Y., Yang, Y., Peng, L. J., Mao, J. Y., Yang, M. R., Tian, Y., & Sheng, J. (2022). Isothiocyanate from Moringa oleifera seeds inhibits the growth and migration of renal cancer cells by regulating the PTP1B-dependent Src/Ras/Raf/ERK signaling pathway. *Frontiers in Cell and Developmental Biology*, *9*(January), 1–16. https://doi.org/10.3389/fcell.2021.790618

Zunica, E. R. M., Yang, S., Coulter, A., White, C., Kirwan, J. P., & Gilmore, L. A. (2021). Moringa oleifera seed extract concomitantly supplemented with chemotherapy worsens tumor progression in mice with triple negative breast cancer and obesity. *Nutrients*, *13*(9). https://doi.org/10.3390/nu13092923