

Mangifera indica (Mango): A Promising Medicinal Plant for Breast Cancer Therapy and Understanding Its Potential Mechanisms of Action

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Breast Cancer: Targets and Therapy

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REVIEW

Mangifera indica (Mango): A Promising Medicinal Plant for Breast Cancer Therapy and Understanding Its Potential Mechanisms of Action

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Kah Min Yap[1]
Mahendran Sekar[1]
Lay Jing Seow[1]
Siew Hua Gan[2]
Srinivasa Reddy Bonam[3]
Nur Najihah Izzati Mat Rani[4]
Pei Teng Lum[1]
Vetriselvan Subramaniyan[5]
Yuan Seng Wu[5]
Neeraj Kumar Fuloria[6]
Shivkanya Fuloria[6]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, Ipoh, 30450, Perak, Malaysia; [2]School of Pharmacy, Monash University Malaysia, Bandar Sunway, 47500, Selangor Darul Ehsan, Malaysia; [3]Institut National de la Santé et de la Recherche Médicale; Centre de Recherche des Cordeliers, Equipe-Immunopathologie et Immunointervention Thérapeutique, Sorbonne Université, Université de Paris, Paris, France; [4]Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, Ipoh, 30450, Perak, Malaysia; [5]Faculty of Medicine, Bioscience and Nursing, MAHSA University, Selangor, 42610, Malaysia; [6]Faculty of Pharmacy, AIMST University, Kedah, 08100, Malaysia

Abstract: Globally, breast cancer is the most common cancer type and is one of the most significant causes of deaths in women. To date, multiple clinical interventions have been applied, including surgical resection, radiotherapy, endocrine therapy, targeted therapy and chemotherapy. However, 1) the lack of therapeutic options for metastatic breast cancer, 2) resistance to drug therapy and 3) the lack of more selective therapy for triple-negative breast cancer are some of the major challenges in tackling breast cancer. Given the safe nature of natural products, numerous studies have focused on their anti-cancer potentials. *Mangifera indica*, commonly known as mango, represents one of the most extensively investigated natural sources. In this review, we provide a comprehensive overview of *M. indica* extracts (bark, kernel, leaves, peel and pulp) and phytochemicals (mangiferin, norathyriol, gallotannins, gallic acid, pyrogallol, methyl gallate and quercetin) reported for in vitro and in vivo anti-breast cancer activities and their underlying mechanisms based on relevant literature from several scientific databases, including PubMed, Scopus and Google Scholar till date. Overall, the in vitro findings suggest that *M. indica* extracts and/or phytochemicals inhibit breast cancer cell growth, proliferation, migration and invasion as well as trigger apoptosis and cell cycle arrest. In vivo results demonstrated that there was a reduction in breast tumor xenograft growth. Several potential mechanisms underlying the anti-breast cancer activities have been reported, which include modulation of oxidative status, receptors, signalling pathways, miRNA expression, enzymes and cell cycle regulators. To further explore this medicinal plant against breast cancer, future research directions are addressed. The outcomes of the review revealed that *M. indica* extracts and their phytochemicals may have potential benefits in the management of breast cancer in women. However, to validate its utility in the creation of innovative and potent therapeutic agents to treat breast cancer, more dedicated research, especially clinical studies are needed to explore the anti-breast cancer potentials of *M. indica* extracts and their phytochemicals.

Keywords: mango, *Mangifera indica*, mangiferin, breast cancer, molecular mechanism, natural products, women's health

Introduction

Globally, breast cancer remains one of the most common cancers and causes of deaths in females.[1] In 2020 alone, there were 2,261,419 new cases and 684,996 deaths globally.[2] In fact, most breast cancer cases are diagnosed at early stages when the disease is deemed as relatively curable.[3] However, approximately 20–30% of these patients suffer from distant relapse with cancer cells spreading from the primary site to

Correspondence: Mahendran Sekar
Tel +6016 – 3346653
Fax +605 – 2536634
Email mahendransekar@unikl.edu.my

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distant body parts (eg, bones, brain, distant nodal, liver, lungs and pleural/peritoneal metastases).[3]

The development of breast cancer is thought to be related to several important risk factors; with aging playing the major role, followed by family history (eg, *BRCA1/2* mutations), reproductive factors (eg, early menarche and late menopause), hormonal imbalances (eg, elevated estrogen levels) and lifestyle (eg, high fat intake and alcohol consumption).[4] In fact, only 5–10% of breast cancer cases are thought to have a genetic background, with *BRCA1/2* germline mutations contributing to approximately 50% of these hereditary cases.[5,6]

Breast cancer is a heterogenous disease owing to the existence of molecular and phenotypic variations within a patient's tumor (intra-tumoral heterogeneity) and between different patients' tumors (inter-tumoral heterogeneity).[7] Based on immunohistochemistry and gene expression profiling, breast cancer can be classified into different biological subtypes.[8] The main subtype classification system is gene expression profile-based by which tumors are classified into 1) the estrogen receptor (ER)-positive (ie, luminal A and B) group (70%), 2) the human epidermal growth factor receptor 2 (HER2)-enriched group (15–20%) or 3) the basal-like [or triple-negative; ER-, progesterone receptor (PR)- and HER2-negative] group (15%).[9] Such subtype classification is clinically important in informing treatment options since these subtypes demonstrate variations in their prognosis and treatment responses (Table 1).[9,10] Although endocrine therapy is usually effective in most patients with ER-positive tumors, 15–20% and 30–40% of all ER-positive tumors exhibit intrinsic and acquired resistance to the therapy, respectively.[11] Triple-negative breast cancer, the subtype known to possess a more aggressive nature, particularly lacks targeted therapy and is reliant on chemotherapy that is non-specific,[12] thus creating the need for more selective and effective agents for breast cancer treatment.

Natural products are an important source for the discovery of new anti-cancer agents that may provide long-term cancer control with minimal side effects.[13] Isolation of paclitaxel from the bark of Pacific yew trees (*Taxus brevifolia*) or vinblastine and vincristine from the leaves of Madagascar periwinkle plants (*Catharanthus rosea*) are some of the many examples.[14] Fish, green tea, medicinal plants and fruits have been explored for their potentials in breast cancer treatment, with *Mangifera indica* (*M. indica*) being one example.[15]

M. indica, commonly known as mango, belongs to the Anacardiaceae flowering plant family.[16] Although initially thought to have originated from India, *M. indica* has been widely cultivated in Southeast Asia.[16] Different *M. indica* plant parts contain varying types and quantities of phytochemicals[17] (Table 2), and they have traditionally been exploited for the treatment of several medical conditions, including gastrointestinal, genitourinary, ophthalmic and respiratory conditions.[18,19] Preclinical studies on the extracts prepared from various plant parts have demonstrated anti-cancer, anti-inflammatory, antimicrobial, antioxidant and immunomodulatory activities.[16,20–23] Specifically, various studies have reported on the anti-cancer activities of *M. indica* pulp extracts in breast cancer,[24–26] thus suggesting the consumption of *M. indica* fruits may potentially be beneficial in breast cancer management. Further, the phytochemical profiling of *M. indica* extracts has suggested that their anti-cancer activities are attributed mainly to the polyphenolic contents.[27] Therefore, the phytochemicals have the potentials to be developed as anti-cancer drugs.

This review described preclinical studies conducted on various *M. indica* extracts and phytochemicals (Figure 1) against breast cancer. The proposed mechanisms for their anti-breast cancer activity were then discussed in detail. Although *M. indica* or its derived products have not been evaluated in clinical studies for breast cancer treatment, they have been clinically evaluated for central nervous system activities,[28] chronic constipation,[29] gastrointestinal and upper respiratory tract infections[30] and rheumatoid arthritis,[31] etc. Hence, future research directions are addressed in order to further explore this medicinal plant and its phytochemicals against breast cancer.

Table 1 Typical Systemic Therapeutic Options for the Three Major Molecular Subtypes of Breast Cancer

	Luminal ER-Positive	HER2-Enriched	Basal-Like (or Triple-Negative)
Endocrine therapy	For all patients	For patients with ER-positive tumors	N/A
HER2-targeted therapy	N/A	For all patients	N/A
Chemotherapy	For certain patients	For all patients	For all patients

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2.

Table 2 Type of Compounds Identified from Different Parts of *M. indica*

M. indica parts	Types of Extract	Compounds	References
Bark	Water	**Polyphenols** Flavan-3-ols [(+)-catechin and (-)-epicatechin] Phenolic acids (gallic acid and 3,4-dihydroxybenzoic acid) Phenolic esters (methyl gallate, propyl gallate and propyl benzoate) Xanthones (mangiferin) **Fatty acids** **Microelements** **Steroids** **Terpenoids**	Rodeiro et al (2007)[32] Núñez Sellés et al (2002)[33]
Kernel	Ethanol	1-Butanol, 3-methyl-, acetate Butane,1,1-diethoxy-3-methyl- Propane,1,1,3-triethoxy- Ethaneperoxoic acid, 1-cyano-1-(2-methylphenyl) ethyl ester Apigenin 7-glucoside Disperse Red 11 Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl- (butylated hydroxytoluene, BHT) Chlorazanil Isoheptadecanol (1-hexadecanol,2-methyl) cis-5-Dodecenoic acid, (3-cyanopropyl) dimethylsilyl ester Fumaric acid, 2-decyl undecyl ester Phthalic acid, hept-2-yl isohexyl ester	Abdullah et al (2014)[39]
Leaves	Ethanol 50% CO_2:25% ethanol:25% water 50% ethanol:50% water 50% ethanol:50% water fraction previously extracted with supercritical-CO_2	Gallic acid 3,4-Dihydroxybenzoic acid Maclurin glucoside Methyl gallate Iriflophenone 3-C-β-D-glucoside Iriflophenone 3-C-(2-*O*-*p*-hydroxybenzoyl)-β-D-glucoside Mangiferin Homomangiferin Iriflophenone 3-C-(2-6-di-*O*-galloyl)-β-D-glucoside Iriflophenone 3-C-(2-*O*-galloyl)-β-D-glucoside *tetra*-*O*-galloyl-glucose Quercetin 3-D-galactoside Quercetin 3-β-D-glucoside Quercetin 3-*O*-xyloside Quercetin 3-*O*-α-L arabinopyranoside Penta-*O*-galloyl-glucose	Fernández-Ponce et al (2017)[27]

(*Continued*)

Table 2 (Continued).

M. indica parts	Types of Extract	Compounds	References
Pulp	Homogenisation of pulp with ethanol: methanol: acetone (1:1:1) mixture and combining of both C_{18}-bounded and ethyl acetate fractions of the resulting extract	Gallic acid Gallotannins Mono-galloyl glucoside *tetra*-Galloyl glucoside *penta*-Galleo glucoside OH-benzoic acid hexoside	Banerjee et al (2015)[24]
Peel and pulp	Methanol	**Phenolic acids** Gallic acid and derivatives (methyl gallate, methyl galloyl gallate and digallic acid) **Flavonoids** Quercetin and derivatives Kaempferol Mangiferin and isomers Epicatechin-3-*O*-gallate **Fatty acids and derivatives**	Pierson et al (2014)[54]
Peel	Ethanol	Chlorogenic acid Caffeic acid Catechin 3,4-Dicaffeoyl quinic acid 4,5-Dicaffeoyl quinic acid Gallic acid Quercetin	Shaban et al (2016)[51]

Methods

Relevant literature was collected from several scientific databases, including PubMed, Scopus and Google Scholar. The literature search was achieved using the following keywords: "Mango OR *Mangifera indica* OR Mangiferin AND Breast Cancer". Papers not written in the English language or without abstracts were excluded from initial screening. Any duplicate was also excluded. Following complete screening and selection of appropriate articles, the yielded information was summarised for 1) the anti-cancer activities of *M. indica* extracts or their phytochemicals in in vitro and in vivo breast cancer models and 2) the potential mechanisms underlying the bioactivities. All key findings from the selected articles were summarised in Table 3 and detailed in the following sections.

Effect of Extracts of Various *M. indica* Parts Against Breast Cancer

Bark Extract of *M. indica*

Vimang is an aqueous *M. indica* bark extract consisting of a mixture of polyphenols, fatty acids (FAs), microelements, steroids and terpenoids.[32,33] It is a registered anti-inflammatory phytomedicine used either as cream, tablets or syrup in Cuba, where its industrial-scale production has been carried out.[34,35] Vimang has shown various pharmacological activities such as analgesic, anti-cancer, anti-inflammatory, antioxidant and immunomodulatory activities.[23,36,37] One of its popular uses in Cuba is to improve the quality of life of cancer patients, thus suggesting its potential use in cancer treatment.[38] A study showed that Vimang can significantly inhibit the proliferation of MDA-MB-231 (triple-negative) cells in a dose-dependent manner, with half-maximal inhibitory concentration (IC_{50}) at 259 μg/mL.[34]

Kernel Extract of *M. indica*

A relevant study reported the ability of *M. indica* kernel extract to induce a dose-dependent reduction in the viability of MCF-7 (luminal A; ER-positive, PR-positive, HER2-negative), MDA-MB-231 and MCF-10A (normal human mammary epithelial cell line) cells.[39] The IC_{50} values of kernel extract were significantly lower for the cancer cells (15 μg/mL for MCF-7 and 30 μg/mL for MDA-MB-231 cells) than the normal cells (149 μg/mL),

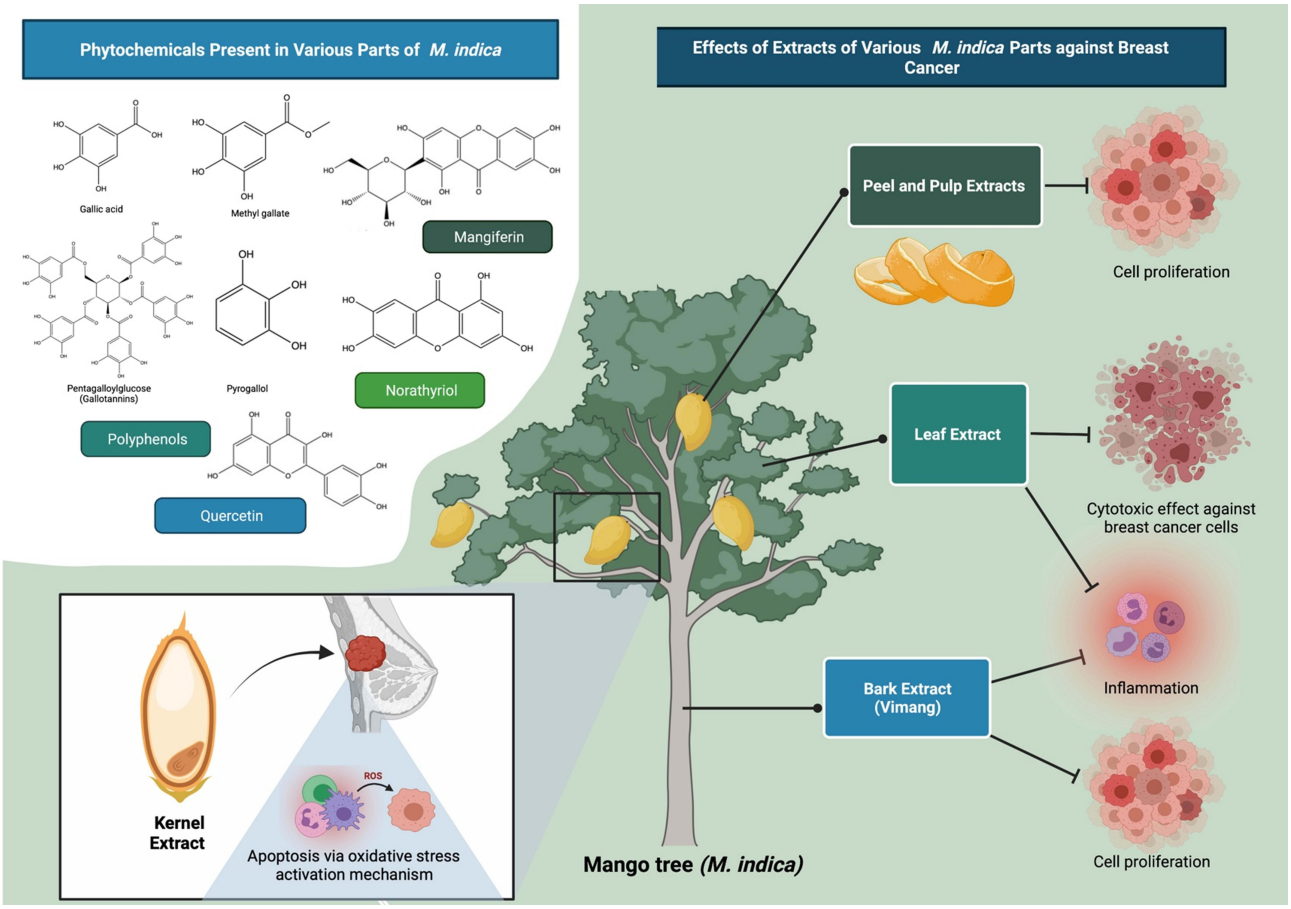


Figure 1 An overview of effects of *M. indica* extracts and their phytochemicals against breast cancer. Extracts of various *M. indica* parts (bark, kernel, leaves, peel and pulp) have been used to treat a variety of medical problems including breast cancer; as these plant parts contain varying types and amounts of polyphenols, some of which possess anti-proliferative and pro-apoptotic activities. According to phytochemical profile analysis, the anti-cancer activity of *M. indica* extracts is mostly attributable to the presence of polyphenolic compounds such as mangiferin, gallotannins, gallic acid, pyrogallol, methyl gallate and quercetin.

suggesting that the kernel extract is cancer cell-selective.[39] Subsequent phytochemical profiling of the extract detected 12 major compounds,[39] some of which have demonstrated anti-breast cancer potentials in previous studies.[40–47]

Leaf Extract of *M. indica*

An emerging body of evidence has suggested the association between chronic inflammation and cancer risk.[48] *M. indica* leaf tea has traditionally been used to ameliorate inflammation.[27] The polyphenols believed to be the major contributors to *M. indica*'s anti-cancer activity are present mainly in the bark and leaves.[49] Nevertheless, to date, cancer-related studies have generally been focussed on *M. indica* bark, peel and pulp extracts. A study revealed the cytotoxic effect of ethanolic *M. indica* leaf extract (200 µg/mL) on BT-474 (luminal B; ER-/PR-/HER2-positive) cells.[50] In another study, it was observed that while PEW (50% ethanol:50% water) and FEW (50% ethanol:50% water fraction previously extracted with supercritical carbon dioxide) extracts (0.01–10 µg/mL) with a higher methyl gallate and homomangiferin content are more cytotoxic to MDA-MB-231 cells, PET (pure ethanol) and CEW (50% carbon dioxide:25% ethanol:25% water) extracts (0.01–0.1 µg/mL) with a higher gallotannin content are more cytotoxic to MCF-7 cells.[27] This observation suggests that phytochemicals can influence the selectivity of extracts towards different subtypes of breast cancer cells.[27] Additionally, PET, CEW, PEW and FEW extracts caused only slight reduction in the viability of MCF-10 cells, suggesting that the leaf extracts are cancer cell-selective.[27]

Peel and Pulp Extracts of *M. indica*

The anti-breast cancer activities of *M. indica* peel and pulp extracts have been more frequently reported compared to the other plant parts. García-Solís et al[25] reported the ability of aqueous *M. indica* pulp extract (4% v/v) to

Table 3 A Summary of in vitro and in vivo Studies of *M. indica* Extracts and Their Phytochemicals Evaluated for Anti-Breast Cancer Potentials

Extracts/ Phytochemicals	Cell Lines/Animals (Sex)	Observations	Mechanism of Action	References
Bark	MDA-MB-231 cell line	↓ Cell proliferation	Inhibition of NFκB signalling pathway	García-Rivera et al (2011)[34]
Kernel	MCF-7 cell line MDA-MB-231 cell line	↓ Cell viability	N/A	Abdullah et al (2014)[39]
	MCF-7 cell line	↑ ROS and MDA levels ↓ GSH level ↑ p53 level ↑ Pro-apoptotic markers (Bax and cytochrome c) ↓ Pro-survival markers (Bcl-2) ↑ Caspases 7, 8 and 9 activities	Activation of oxidative stress-induced cell death	Abdullah et al (2015a)[160]
	MDA-MB-231 cell line	↑ ROS and MDA levels ↓ GSH level ↑ p53 level ↑ Pro-apoptotic markers (Bax and cytochrome c) ↓ Pro-survival markers (Bcl-2) ↑ Caspases 3, 8 and 9 activities	Activation of oxidative stress-induced cell death	Abdullah et al (2015b)[161]
Leaves	BT-474 cell line	↓ Cell viability	N/A	Ganogpichayagrai et al (2017)[50]
	MCF-7 cell line MDA-MB-231 cell line	↓ Cell viability	Antioxidant activity	Fernández-Ponce et al (2017)[27]
Pulp	MCF-7 cell line	↓ Cell proliferation	Antioxidant activity	García-Solís et al (2009)[25]
	BT-474 cell line Mice bearing BT-474 xenografts (female)	↓ Cell proliferation ↓ Tumor volume	Suppression of PI3K/AKT pathway Induction of miR-126 expression	Banerjee et al (2015)[24]
Peel and pulp	MCF-7 cell line	↓ Cell viability	Modulation of PPARs	Wilkinson et al (2011)[26]
	MCF-7 cell line MDA-MB-231 cell line	↓ Cell viability (peel extract only) ↑ Cell death (peel extract only)	N/A	Hoang et al (2015)[52]
	MCF-7 cell line	↓ Cell viability (peel extract only)	Modulation of intracellular Ca^{2+} signalling	Taing et al (2015)[53]
Peel	MCF-7 cell line	↓ Cell viability	Antioxidant activity Inhibition of aromatase enzymatic activity and expression	Shaban et al (2016)[51]
Polyphenols	MDA-MB-231 cell line	↓ Cell growth	Antioxidant activity	Noratto et al (2010)[55]
	MCF10DCIS cell line	↓ Cell proliferation	Suppression of PI3K/AKT pathway	Nemec et al (2016)[56]
	Mice bearing MCF10DCIS xenografts (female)	↓ Tumor volume	Suppression of PI3K/AKT pathway Activation of AMPK signalling pathway	Nemec et al (2017)[57]

(*Continued*)

Table 3 (Continued).

Extracts/ Phytochemicals	Cell Lines/Animals (Sex)	Observations	Mechanism of Action	References
Mangiferin	MCF-7 cell line T47D cell line MDA-MB-231 cell line BT-549 cell line Mice bearing MDA-MB-231 xenografts (female)	↓ Cell proliferation ↑ Apoptosis ↓ Cell migration ↓ Cell invasion ↓ Tumor volume ↓ Tumor weight	Inactivation of β-catenin pathway	Li et al (2013)[66]
	MCF-7 cell line MDA-MB-231 cell line	↓ Cell viability ↓ Cell migration ↓ Cell invasion	Inhibition of Rac1/WAVE2 signalling pathway	Deng et al (2018)[67]
	MCF-7 cell line	↓ Cell viability ↓ Proliferation marker (PCNA) ↓ Pro-survival markers (Bcl-2) ↑ Pro-apoptotic markers (Bax, caspase 9 and p27) ↓ Cell migration	Inhibition of HMG-CoA reductase, proteasome and plasmin enzymatic activities	Cuccioloni et al (2016)[68]
	MCF-7 cell line	↓ Cell viability	N/A	Louisa et al (2014)[74]
	MDA-MB-231 cell line	↔ Cell viability	Inhibition of NFκB signalling pathway	García-Rivera et al (2011)[34]
	MCF-7 cell line	↔ Cell viability	Modulation of ER activity	Wilkinson et al (2015)[75]
	MCF-7 cell line	N/A	Inhibition of NFκB signalling pathway	Sarkar et al (2004)[228]
Norathyriol	MCF-7 cell line	↓ Cell viability	Modulation of ER activity	Wilkinson et al (2015)[75]
Gallotannin (5GG)	MCF-7 cell line	↓ Cell growth	Suppression of PI3K/AKT pathway Modulation of ER activity	Hua et al (2006)[94]
	MCF-7 cell line	↓ Cell growth ↑ G1-phase arrest	Modulation of cell cycle regulators	Chen et al (2003)[95]
Gallic acid	MDA-MB-231 cell line	↓ Cell viability	Inhibition of NFκB signalling pathway	García-Rivera et al (2011)[34]
	MCF-7 cell line	↓ Cell proliferation ↑ S-phase and G2/M-phase arrest	Modulation of cell cycle regulators	Hsu et al (2011)[111]
	MCF10DCIS cell line	↓ Cell proliferation	N/A	Nemec et al (2016)[56]
	MCF-7 cell line	↓ Cell growth ↑ Apoptosis ↑ Fas, FasL levels and caspase 8 activity ↓ $\Delta\Psi_m$ ↑ Bax/Bcl-2 ratio, cytochrome c level and caspase 9 activity ↑ Caspase 8-mediated Bid cleavage	N/A	Ke Wang et al (2014)[112]

(*Continued*)

Table 3 (Continued).

Extracts/ Phytochemicals	Cell Lines/Animals (Sex)	Observations	Mechanism of Action	References
Pyrogallol	MCF10DCIS cell line	↓ Cell proliferation	Suppression of PI3K/AKT pathway	Nemec et al (2016)[56]
	Mice bearing MCF10DCIS xenografts (female)	↓ Tumor volume	Suppression of PI3K/AKT pathway Activation of AMPK signalling pathway	Nemec et al (2017)[57]
Methyl gallate	MCF10DCIS cell line	↓ Cell proliferation	N/A	Nemec et al (2016)[56]
Quercetin	MCF-7 cell line	↓ Cell viability	Modulation of ER activity	Wilkinson et al (2015)[75]
	MCF-7 cell line	↓ Cell growth ↑ Apoptosis ↑ S-phase arrest	Activation of oxidative stress-induced cell death	Zhang et al (2012)[147]
	MCF-7 cell line	↓ Cell viability ↑ Apoptosis ↑ S-phase arrest ↑ ATF6, GRP78, PERK and GADD153 levels ↓ $\Delta\Psi_m$ ↑ AIF, Bid, caspase 6, caspase 8, caspase 9, Fas and TRAIL levels ↓ Bcl-2, PARP and XIAP levels ↑ Caspases 6, 8 and 9 activities ↑ AIF and GADD153 nuclear translocation	Modulation of intracellular Ca^{2+} signalling Modulation of cell cycle regulators	Chou et al (2010)[148]
	MDA-MB-231 cell line	↓ Cell viability ↑ Apoptosis ↑ G2/M-phase arrest ↑ ATF6-α, GRP78 and PERK levels ↓ Pro-caspase 3 ↓ $\Delta\Psi_m$ ↑ Bax, caspase 3, caspase 8, cytochrome c and Fas levels ↓ PARP and XIAP levels ↑ Caspases 3, 8 and 9 activities ↑ AIF level and mitochondrio-nuclear translocation	Modulation of intracellular Ca^{2+} signalling Modulation of cell cycle regulators	Chien et al (2009)[149]
	MDA-MB-231 cell line Mice bearing MDA-MB-231 xenografts (female)	↓ Cell proliferation ↑ Apoptosis ↑ G2/M-phase arrest ↑ Caspases 3 and 7 activities ↓ Cell migration ↓ Tumor growth	Suppression of PI3K/AKT pathway Activation of AMPK signalling pathway	Rivera Rivera et al (2016)[150]

Notes: ↑ increased; ↓ decreased; ↔ no significant change.

Abbreviations: AIF, apoptosis-inducing factor; AMPK, AMP-activated protein kinase; ATF6-α, AMP-dependent transcription factor 6-alpha; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; Bid, BH3-interacting domain death agonist; Ca^{2+}, calcium; ER, estrogen receptor; Fas, Fas cell surface death receptor; FasL, Fas ligand; GADD153, growth arrest and DNA damage 153; GRP78, 78-kDa glucose-regulated protein; GSH, glutathione; HMG-CoA reductase, 3-hydroxy-3-methyl-glutaryl-coenzyme A; MDA, malondialdehyde; miR-126, microRNA-126; $\Delta\Psi_m$, mitochondrial membrane potential; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PARP, Poly-ADP ribose polymerase; PCNA, proliferating cell nuclear antigen; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PI3K/AKT, phosphoinositide 3-kinase/protein kinase B; PPARs, peroxisome proliferator-activated receptors; ROS, reactive oxygen species; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; XIAP, X-linked inhibitor of apoptosis protein; 5GG, pentagalloylglucose.

induce a significant reduction in MCF-7 cell proliferation. In other studies, Wilkinson et al[26] and Shaban et al[51] observed a significant reduction in MCF-7 cell viability following treatment with ethyl acetate fractions prepared from *M. indica* peels and pulps as well as ethanolic *M. indica* peel extract, respectively. On the other hand, Banerjee et al[24] reported the ability of *M. indica* pulp extract (2.5–20.0 mg GAE/L) to dose-dependently inhibit BT-474 cell proliferation. In the same study, *M. indica* pulp extract (0.8 mg GAE/day; 35 days) also significantly reduced tumor volume in nude mice bearing BT-474 xenografts.

As different *M. indica* varieties have different phytochemical profiles and bioactivities, two studies have evaluated the anti-breast cancer activities of methanolic peel and pulp extracts prepared from three *M. indica* varieties (Irwin, Kensington Pride and Nam Doc Mai).[52,53] Both studies consistently reported Nam Doc Mai peel extract as the only extract with significant cytotoxicity against MCF-7 cells. Additionally, only Nam Doc Mai peel extract was observed to possess significant cell-death inducing activity in MDA-MB-231 cells.[52] Previous phytochemical profiling of *M. indica* peel and pulp extracts revealed that Nam Doc Mai peel extract uniquely contained gallic acid and galloylated derivatives as well as a higher level of methyl gallate,-[54] which may explain its higher bioactivities.

Effect of Phytochemicals from *M. indica* Against Breast Cancer

Polyphenols

The anti-cancer activity of *M. indica* has frequently been attributed to its polyphenolic content. Noratto et al[55] reported that the polyphenolic extracts prepared from different *M. indica* varieties suppressed MDA-MB-231 cell growth in a dose-dependent manner. In another study conducted by Nemec et al,[56] a low molecular weight fraction of *M. indica* polyphenols (1 mg/L) was found to significantly reduce the proliferation of MCF10DCIS [a ductal carcinoma in situ (DCIS) model cell line] cells. In the same study, combined treatment with *M. indica* polyphenols and a conventional anti-cancer agent [5-fluorouracil (5-FU)] demonstrated a greater anti-proliferative effect on MCF10DCIS cells than either treatment alone, which is suggestive of a synergistic effect. The same research group did a follow-up study on the in vivo anti-cancer effects of *M. indica* polyphenols (0.8 mg GAE/day; 4 weeks) in nude mice carrying MCF10DCIS xenografts, whereby a reduction in tumor volume (>70%) was observed following treatment.[57]

Various studies have investigated into the anti-breast cancer activities of individual *M. indica* polyphenols, instead of *M. indica* polyphenolic mixtures, as described below.

Mangiferin

Mangiferin (1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside), a plant natural xanthonoid, is the predominant phytochemical found in various *M. indica* parts, including bark, fruits, leaves and roots.[58,59] Mangiferin has been reported to exhibit various pharmacological activities such as analgesic, anti-bacterial, anti-cancer, anti-diabetic, anti-inflammatory, antioxidant and immunomodulatory activities.[59–64] Importantly, mangiferin has shown protective effects against various types of cancers, including breast cancer.[65]

As reported by Li et al,[66] mangiferin (75–300 μM) caused a dose-dependent reduction in the proliferation of ER-positive (MCF-7 and T47D) and ER-negative (MDA-MB-231 and BT-549) breast cancer cells, which may be attributed to an increase in apoptosis. In the same study, mangiferin (12.5–50.0 μM) also demonstrated dose-dependent anti-migratory and anti-invasive effects on highly metastatic MDA-MB-231 and BT-549 cells. When the in vivo anticancer activity of mangiferin (100 mg/kg/day; 5 weeks) was evaluated in severe-combined immunodeficiency (SCID) mice bearing MDA-MB-231 xenografts, significant reduction in both tumor volume and tumor weight was observed.[66]

The cytotoxic, anti-migratory and anti-invasive effects of mangiferin (10–50 μM) have similarly been reported in another study by Deng et al,[67] whereby a dose-dependent reduction in the viability of MCF-7 and MDA-MB-231 cells as well as a significant reduction in the migration and invasion of MDA-MB-231 cells were observed. Notably, mangiferin has also been reported by Cuccioloni et al[68] to be cancer cell-selective, as evidenced by the observations of greater decreases in cell viability, proliferating cell nuclear antigen (PCNA; a marker of cell proliferation) level and anti-apoptotic protein [B-cell lymphoma 2 (Bcl-2)] level as well as greater increases in pro-apoptotic protein [Bcl-2-associated X protein (Bax), caspase 9 and p27] levels in MCF-7 cells than MCF-10A cells following treatment. The same study also showed that mangiferin could suppress plasmin-induced increase in MCF-7 and MCF-10A cell migration.

Doxorubicin, an anthracycline drug, has been used routinely for the treatment of various cancers, including breast cancer.[69] However, its use has been limited mainly due to cardiotoxicity and resistance.[70] One of the causes of doxorubicin resistance is the increased expression of drug efflux pumps such as P-glycoprotein (Pgp), multidrug resistance–associated protein-1 (MRP-1) and breast cancer resistance protein (BCRP).[71–73] Louisa et al[74] found that mangiferin can increase the sensitivity of MCF-7 cells (pre-treated with doxorubicin) towards doxorubicin treatment, as evidenced by a significant reduction in the viability of MCF-7 cells treated with mangiferin (10–50 μM) in combination with doxorubicin. Subsequent evaluation of Pgp, MRP1 and BCRP gene expressions showed that while high dose (50 μM) mangiferin could significantly downregulate Pgp, there were no significant changes in the expressions of MRP1 and BCRP at all doses.[74] These findings thus suggest that high-dose mangiferin can potentially serve as a chemosensitizer for doxorubicin by downregulating Pgp expression.[74]

In contrast to the above-mentioned studies, a number of studies have failed to observe the anti-breast cancer activity of mangiferin,[34,75] thus warranting further investigation. In one of the studies, Wilkinson et al[75] showed that mangiferin is capable of activating ERα but not ERβ, and suggested that the observed lack of anti-proliferative activity of mangiferin may be linked to the differential activation or heterodimer formation of ERα and ERβ.

Norathyriol

Norathyriol (1,3,6,7-tetrahydroxyxanthone), an aglycone derivative of mangiferin, is structurally similar to mangiferin except for a C-glucosyl bond.[76] Ample of evidence has shown that orally consumed mangiferin is firstly subjected to de-glycosylation by intestinal bacterium to form norathyriol prior to being absorbed by the colon, suggesting norathyriol is likely to contribute to the observed bioactivities of mangiferin.[77,78] Studies focussing on norathyriol have reported a range of pharmacological activities.[79–83] For example, a study showed that norathyriol (100 μM) significantly reduced the viability of MCF-7 cells.[75]

Gallotannins, Gallic Acid, Pyrogallol and Methyl Gallate

M. indica is a rich source of gallotannins and lower molecular weight gallates such as gallic acid.[56] Although orally consumed gallotannins are un-absorbable owing to their high molecular weight, they can be hydrolysed in the gastrointestinal tract by tannase to release free gallic acid, which can subsequently undergo decarboxylation to pyrogallol via the activity of gallic acid decarboxylase in intestinal microbiota.[84,85] Gallotannins have been reported to exhibit a wide range of pharmacological activities, including anti-cancer,[86–88] anti-diabetic,[89] anti-inflammatory,[90] antimicrobial,[91] and antioxidant[92] activities. Pentagalloylglucose (1,2,3,4,6-penta-*O*-galloyl-β-D-glucose; 5GG), a gallotannin that can be found in *M. indica*, has been investigated for its effects on breast cancer cell lines.[93] For instance, treatment with 5GG (0.5–80 μM) was reported to exhibit growth-inhibitory effect on MCF-7 cells in both time- and dose-dependent manner.[94] Another study similarly reported the dose-dependent growth-inhibitory effect of 5GG (1–100 μM) on MCF-7 cells.[95] Further cell cycle analysis revealed a dramatic increase in G1-phase cell number, suggesting that 5GG can induce a G1-phase arrest in MCF-7 cells.[95]

Gallic acid (3,4,5-trihydroxybenzoic acid), one of the most abundantly distributed plant phenolic compounds that can be found in certain fruits and medicinal plants, has been used extensively in both food and pharmaceutical industries.[96] Gallic acid has demonstrated various pharmacological activities.[97–101] Its anti-cancer activity, in particular, has been extensively studied in various cancer types.[102–110] In a study, MDA-MB-231 cells treated with gallic acid showed a significant dose-dependent reduction in cell viability, with IC_{50} at 10 μg/mL.[34] Another study reported the ability of gallic acid (1–10 or 2–12 μg/mL) to induce a reduction in cell proliferation and an increase in S-phase and G2/M-phase ratios in MCF-7 cells in a dose-dependent manner.[111] Similarly, the anti-proliferative activity of gallic acid (1mg/L) has also been demonstrated in MCF10DCIS cells.[56]

Of note, Ke Wang et al[112] observed a dose-dependent reduction in MCF-7 cell growth following gallic acid treatment (IC_{50}: 80.5 μM) and attributed this effect to apoptosis induction. Subsequent analyses revealed increased Fas cell surface death receptor (Fas) and Fas ligand (FasL) protein levels and caspase 8 activity, suggesting the induction of death receptor apoptotic pathway.-[112] Reduced mitochondrial membrane potential ($\Delta\Psi_m$) as well as increased Bax/Bcl-2 ratio, cytosolic cytochrome c level and caspase 9 activity were also observed, which was suggestive of the induction of mitochondrial apoptotic pathway.[112] Moreover, caspase 8-mediated Bid cleavage, which is known to be followed by cytochrome c release and caspase 9 activation, was observed in gallic acid-

treated MCF-7 cells.[112] Collectively, these findings suggest that gallic acid-induced apoptosis of MCF-7 cells involved both mitochondrial and death receptor pathways as well as a cross-link between the two pathways.[112]

Pyrogallol (1,2,3-trihydroxybenzene), a microbial metabolite of *M. indica* gallotannins, can also be found naturally in many other fruits and vegetables.[113] Pyrogallol is used commercially mainly in the production of pharmaceuticals and pesticides.[114] Similar to gallotannins and gallic acid, pyrogallol has been found to possess multiple pharmacological activities[113,115,116] in addition to anti-carcinogenic properties in various cancer types.[117–120] For instance, pyrogallol (1 mg/L) has been reported to exert anti-proliferative effect on MCF10DCIS cells.[56] However, pyrogallol at a higher dose (10 mg/L) was observed to have insignificant cytotoxicity against normal human breast epithelial cells MCF-12F, suggesting its selectivity of action towards cancer cells.[56] The same research group then studied the in vivo effect of pyrogallol, and found that treatment with pyrogallol (0.2 mg/day; 4 weeks) significantly reduced tumor volume (>70%) in mice bearing MCF10DCIS xenografts.[57]

Methyl gallate (methyl-3,4,5-trihydroxybenzoate), a methyl ester of gallic acid, is naturally found in *M. indica* and various other plants.[121–124] Methyl gallate possesses a wide range of medicinal properties.[122,125–131] For instance, methyl gallate (1 mg/L) was observed to induce a >40% reduction in MCF10DCIS cell proliferation.[56]

Quercetin

Quercetin (3,5,7,3′,4′-pentahydroxyflavone) is a natural flavonoid that can be found abundantly in various fruits and vegetables.[132] For instance, there have been reports of the presence of quercetin in extracts prepared from *M. indica* leaves, peels and pulps.[27,51,54] Quercetin possesses a range of bioactive effects that can potentially be beneficial to human health,[133–139] including its anti-cancer activities in a broad range of cancer types.[140–146]

In a study, Wilkinson et al[75] found that quercetin (100 μM) can significantly reduce MCF-7 cell viability. Another study by Zhang et al[147] reported that quercetin (25–100 μM) can inhibit MCF-7 cell growth in both dose- and time-dependent manner via the induction of apoptosis and S-phase arrest. Chou et al[148] similarly reported the ability of quercetin (10–175 μM) to dose- and time-dependently reduce MCF-7 cell viability via the induction of apoptosis and S-phase arrest. Subsequent analyses revealed increased protein levels of cyclic AMP-dependent transcription factor 6-alpha (ATF6), 78-kDa glucose-regulated protein (GRP78), protein kinase RNA-like endoplasmic reticulum kinase (PERK) and growth arrest and DNA damage 153 (GADD153) in quercetin-treated MCF-7 cells, all of which were suggested to be correlated with endoplasmic reticulum (ER) stress.[148] Furthermore, $\Delta\Psi_m$ loss, increased protein levels of apoptosis-inducing factor (AIF), Bid, caspases 6, 8 and 9, Fas and tumor necrosis factor-related apoptosis inducing ligand (TRAIL), decreased protein levels of Bcl-2, poly-ADP ribose polymerase (PARP) and X-linked inhibitor of apoptosis protein (XIAP), increased activities of caspases 6, 8 and 9 as well as increased nuclear translocation of AIF and GADD153 were also observed, indicating the induction of apoptosis possibly in response to ER stress.[148] Overall, the findings also suggest that quercetin-induced apoptosis in MCF-7 cells is likely to be mediated through both mitochondrial and death receptor pathways.[148]

In another breast cancer cell line (MDA-MB-231 cells), Chien et al[149] also observed a dose- and time-dependent reduction in cell viability following quercetin treatment (IC_{50}: 278 μM). The observed reduction in cell viability can likely be explained by quercetin-induced apoptosis and G2/M-phase arrest,[149] which contrasted the findings by Zhang et al[147] and Chou et al [148] (ie, S-phase arrest in MCF-7 cells). Findings from the subsequent protein expression analysis of various ER stress and apoptotic markers in quercetin-treated MDA-MB-231 cells were similarly suggestive of the occurrence of ER stress-induced apoptosis and the involvement of two caspase-dependent apoptotic pathways (mitochondrial- and caspase 3-dependent pathways).[149] Another study also linked the anti-proliferative effect of quercetin (15 μM) on MDA-MB-231 cells to the induction of apoptosis and G2/M-phase arrest.[150] The same study also showed that quercetin exhibited anti-migratory activity against MDA-MB-231 cells. The researchers then evaluated the in vivo anti-cancer activity of quercetin (15 or 45 mg/kg body weight; thrice weekly for 13 weeks) in SCID mice bearing MDA-MB-231 xenografts, whereby both treatment groups demonstrated significantly reduced (~70%) tumor growth.[150]

Overall Anti-Breast Cancer Mechanisms of Action of *M. indica* Extracts and Their Phytochemicals

Activation of Oxidative Stress-Induced Cell Death

Reactive oxygen species (ROS) are by-products of normal cellular metabolism.[151] They have physiological roles in

signalling pathway (eg, apoptosis, carcinogenesis and proliferation) stimulation but are very transient due to their high reactivity.[152,153] This maintenance of cellular redox balance is critical and is achieved via an antioxidant system.[154] Oxidative stress occurs when ROS production exceeds and depletes the antioxidant defences.[155] The balance of oxidative stress plays an important role in cancer. While moderately elevated ROS levels can contribute to carcinogenesis, cancer cell survival and metastasis, elevated ROS levels above the toxic threshold can cause cancer cell death usually via apoptosis induction.[156–158] In recent years, oxidative stress-induced apoptosis has attracted attentions as a potential anti-cancer mechanism.[159]

In a study conducted by Abdullah et al[160] ethanolic *M. indica* kernel extract was reported to induce both mitochondrial and death receptor pathways of apoptosis in MCF-7 cells via the induction of oxidative stress and the consequent upregulation of p53, as evidenced by the observations of dose- and time-dependent increases in ROS generation and malondialdehyde (MDA; a lipid peroxidation product) level as well as decrease in glutathione (GSH; an antioxidant) level in MCF-7 cells treated with the extracts (5–50 μg/mL). A similar study conducted by the same research group also linked the pro-apoptotic effect of ethanolic *M. indica* kernel extracts on MDA-MB-231 cells to oxidative stress induction and p53 upregulation.[161]

As reported by Zhang et al,[147] the exposure of MCF-7 cells to quercetin (25–100 μM) dose-dependently caused the rightward-shift of dihydroethidium (DHE) signals and increased dichlorofluorescin diacetate (DCFH-DA) signals, which were suggestive of the stimulation of superoxide anion (O_2^-) release and the increased generation of hydrogen peroxide (H_2O_2), respectively. These findings indicate that quercetin is likely to induce apoptosis in MCF-7 cells by increasing oxidative stress.[147]

Antioxidant Activity

Moderately elevated ROS level is considered to be pro-tumorigenic, as ROS function as signalling molecules in various pathways (eg, cell differentiation, proliferation, survival and migration) that can ultimately lead to the promotion of cancer initiation and progression.[162,163] Depleting tumorous cells from these ROS-sensitive signalling pathways via the use of natural antioxidants can therefore potentially be a useful anti-cancer mechanism.[164]

The leaves of *M. indica* are known to contain various families of phenolic compounds that possess potent antioxidant activity.[165] There have been reports that indicate the subcritical water, water, 50% ethanol:50% water, ethanol and 50% carbon dioxide:25% ethanol:25% water extracts of *M. indica* leaves exhibit a more potent antioxidant activity than (+)-α-tocopherol in the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay.[165,166] A study also reported on the antioxidant activities of PET, CEW, PEW and FEW *M. indica* leaf extracts (0.01–100 μg/mL) in non-tumorous (MCF-10A) and tumorous (MCF-7 and MDA-MB-231) cells by detecting changes in intracellular ROS levels, subsequent to the observation of their cytotoxic effects on breast cancer cells.[27] However, the antioxidant activity was only observed at lower extract concentrations.[27] At the highest extract concentration (100 μg/mL), all extracts appeared to be pro-oxidant.[27] The cytotoxic effect observed at this high concentration was thus likely the outcome of oxidative stress-induced cell death.[27]

Studies have also reported on antioxidant activities of *M. indica* peel and pulp extracts. For instance, aqueous *M. indica* pulp extract consistently demonstrated a high antioxidant activity in both DPPH and ferric reducing antioxidant power (FRAP) assays.[25] Another study confirmed the antioxidant activity of ethanolic *M. indica* peel extract in terms of its anti-lipid peroxidation, DPPH radical scavenging, ferric reducing and nitric oxide (NO) radical scavenging activities via the respective use of thiobarbituric acid reaction, DPPH assay, FRAP assay and Griess' reagent.[51]

Besides, the polyphenolic extracts (10 mg GAE/L) of different *M. indica* varieties have also demonstrated antioxidant potentials in the oxygen radical absorbance capacity (ORAC) assay, and a strong correlation between antioxidant activity and total phenolic content has been noted.[55] Figure 2 summarises how *M. indica* extracts and phytochemicals exert their anti-cancer effects by activating oxidative stress-induced cell death or acting as antioxidants.

Modulation of Peroxisome Proliferator-Activated Receptors

Peroxisome proliferator-activated receptors (PPARs), comprising three subtypes (PPARα, PPARβ/δ and PPARγ), are ligand-activated transcription factors belonging to the nuclear hormone receptor superfamily.[167] PPAR activation occurs in response to the binding of endogenous ligands, including Fas, prostacyclins, prostaglandins and triglycerides.[168] By binding the peroxisome proliferator responsive elements (PPREs) upstream of their target

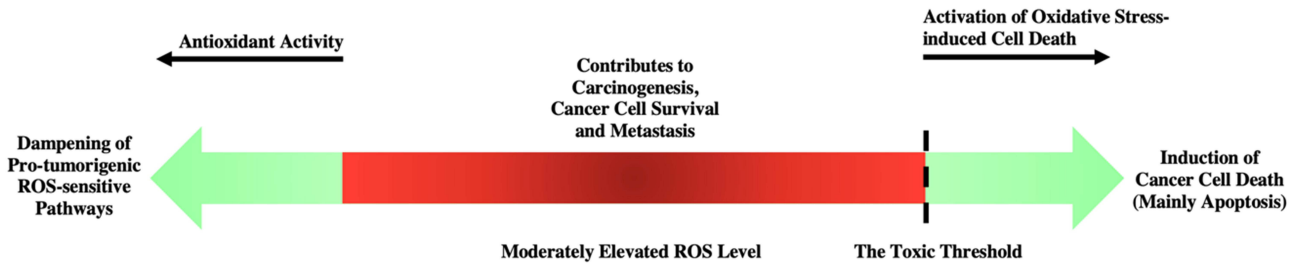


Figure 2 Activation of oxidative stress-induced cell death and antioxidant activity as the anti-cancer mechanisms of *M. indica* extracts and phytochemicals.

genes as a PPAR-retinoid X receptor (RXR) heterodimer, PPARs are able to activate the expressions of genes that are mostly involved in energy homeostasis and metabolic functions.[169]

PPARs have also been implicated in carcinogenesis. Although PPARα has been linked to hepatocarcinogenesis in rodents, several epidemiological studies suggest that this is unlikely to be observed in humans.[170–173] On the other hand, while some studies suggest that PPARδ may contribute to cancer progression,[174–176] other studies suggest that PPARδ may contribute to cancer suppression.[177,178] Similarly, although PPARγ activation has often been linked to proliferation inhibition and apoptosis induction in cancer cells,[179–181] various studies have yielded contradictory results that suggest PPARγ activation may promote cancer development.[182,183]

In a study, Wilkinson et al[26] reported the ability of *M. indica* peel and pulp ethyl acetate fractions to reduce PPAR activity in Cos-7 cells. However, given the controversy surrounding the consequences of PPARγ activation in cancer, whether the PPARγ-inhibitory activity of *M. indica* peel and pulp fractions will be beneficial in breast cancer management requires further evaluation.[26]

Suppression of Phosphoinositide 3-Kinase/Protein Kinase B Pathway

The phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathway can be stimulated in response to the activation of G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) by cytokines, hormones and growth factors.[184] Upon GPCR or RTK activation, PI3K phosphorylates phosphatidylinositol 4, 5-bisphosphate (PIP_2) to form phosphatidylinositol 3, 4, 4-triphosphate (PIP_3) that can, in turn, recruit AKT and phosphoinositide-dependent kinase-1 (PDK-1) to the plasma membrane.[185] Following phosphorylation and activation by PDK-1, the activated AKT can then phosphorylate its downstream substrates, including Bcl-2-associated agonist of cell death (Bad), caspase 9, glycogen synthase kinase-3 (GSK-3), mammalian forkhead transcription factors of the O class (FoxOs), mTOR, NFκB and p21, which have an impact on cell cycle, growth, proliferation and glycometabolism.[184] Mutations and the consequent inappropriate activation of the PI3K/AKT pathway have been observed in many cancer types, contributing to genomic instability, metabolic reprogramming, uncontrolled cell growth and proliferation as well as multidrug resistance.[186–188] In breast cancer, resistance to endocrine therapy, HER2-targeted therapy and cytotoxic chemotherapy has been reported.[189,190] The downregulation of PI3K/AKT signalling may therefore offer benefits in breast cancer management.

M. indica pulp extract has demonstrated inhibitory effect on the PI3K/AKT pathway in both BT-474 cells and mice bearing BT-474 xenografts.[24] In BT-474 cells, *M. indica* pulp extract (2.5–10 mg GAE/L) lowered the messenger ribonucleic acid (mRNA) levels of PI3K, AKT, hypoxia-inducible factor-1 alpha (HIF-1α) and vascular endothelial growth factor (VEGF) as well as the protein levels of phosphorylated PI3K (pPI3K), AKT, phosphorylated AKT (pAKT), NFκB-p65 and VEGF.[24] In BT-474 xenografts, *M. indica* pulp extract (0.8 mg GAE/day; 35 days) downregulated the protein levels of pPI3K, pAKT, NFκB-p65, mTOR, phosphorylated mTOR (pmTOR), HIF-1α and VEGF.[24]

The observed anti-proliferative activities of *M. indica* polyphenols (eg, pyrogallol) in breast cancer cells have also been linked to the downregulation of PI3K/AKT pathway.[56] Nemec et al [56] analysed the mRNA levels of various PI3K/AKT pathway components in MCF10DCIS cells treated with *M. indica* polyphenols or pyrogallol (10 mg/L). Both *M. indica* polyphenols and pyrogallol lowered the mRNA levels of PI3K, mTOR and HIF-1α.[56] However, while *M. indica* polyphenols increased the mRNA level of insulin-like growth factor-1 receptor (IGF-1R) and induced no significant change in the mRNA level of AKT, pyrogallol lowered the mRNA levels of both IGF-1R and AKT.[56] As measuring changes in the

mRNA levels of pathway components provided limited indication on their respective protein activities, the authors also conducted protein expression analysis.[56] A reduction in the total protein levels of IGF-1R, insulin receptor (IR), AKT, mTOR and P70 S6 kinase (p70S6K; a kinase regulated by mTOR) as well as the phosphorylated protein levels of IR, AKT and p70S6K were observed in MCF10DCIS cells following *M. indica* polyphenol treatment.[56] On the other hand, pyrogallol reduced the total protein levels of IGF-1R, IR, insulin receptor substrate-1 (IRS-1), AKT, mTOR and p70S6K as well as the phosphorylated protein levels of IGF-1R, AKT and p70S6K.[56] The same research group similarly reported a suppression of the PI3K/AKT pathway by *M. indica* polyphenols (0.8 mg GAE/day; 4 weeks) and pyrogallol (0.2 mg/day; 4 weeks) in mice bearing MCF10DCIS xenografts, based on findings from mRNA and protein expression analyses.[57]

A study by Hua et al[94] linked the growth-inhibitory effect of 5GG on MCF-7 cells to a suppression of the PI3K/AKT pathway. In the study, it was observed that while 5GG (10–40 μM) had insignificant impact on total AKT protein level, it dose- and time-dependently reduced the phosphorylation of AKT at serine 473 and directly inhibited the activity of AKT. Moreover, 5GG (20 μM) also inhibited epidermal growth factor- and/or estradiol-induced phosphorylation of RTKs upstream of the PI3K/AKT pathway and downregulated the protein levels of several RTKs (EGFR, ErbB2 (or HER2) and ErbB3).[94] In particular, 5GG-induced ErbB2 depletion was blocked by pre-treatment with chloroquine (CQ; a lysosomal inhibitor) but not carbobenzoxy-L-leucyl-L-leucyl-L-leucinal (MG132; a proteasome inhibitor), suggesting 5GG was likely to deplete ErbB2 by promoting its lysosomal degradation.[94] Overall, 5GG can suppress PI3K/AKT signalling by inhibiting AKT and RTK phosphorylation as well as depleting RTKs.[94]

According to Rivera Rivera et al,[150] quercetin (1–15 μM) also exhibits inhibitory activity against the PI3K-AKT pathway, as it is capable of inhibiting the phosphorylation of AKT at serine 473 and the phosphorylation of the downstream effectors of mTOR [p70S6K and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)] in MDA-MB-231 cells. Figure 3 summarises changes in the mRNA, total protein and total phosphorylated protein levels of various PI3K/AKT pathway components induced by *M. indica* extracts and phytochemicals.

Induction of MicroRNA-126 (miR-126) Expression

MicroRNAs (miRNAs) are short non-coding RNAs implicated in the post-transcriptional regulation of eukaryotic gene expression.[191] miRNAs bind to the 3'-untranslated regions of their target mRNAs and, depending on the degree of miRNA-mRNA complementarity, can either result in mRNA degradation or translation inhibition.[192] miRNAs are involved in various cellular processes such as cell development, differentiation, proliferation and apoptosis.[192,193] A reduction in miR-126 expression has frequently been observed in breast cancer, and various studies have reported that a restoration of miR-126 expression can suppress breast cancer cell growth, metastasis and invasion; suggesting miR-126 functions as a tumor suppressor in breast cancer.[194,195] Therefore, the induction of miR-126 expression can be beneficial in treating breast cancer.

A study showed that *M. indica* pulp extract can induce miR-126 expression in BT-474 cells (2.5–10.0 mg GAE/L) and BT-474 xenografts in mice (0.8 mg GAE/day; 35 days).[24] In the same study, the miRNA expression profile screening of extract-treated xenografts further revealed significant changes in the expression of several miRNAs that have a major role in the regulation of tumor cell growth and proliferation. These changes are potentially linked to the observed anti-proliferative effect of *M. indica* pulp extract.[24]

Inhibition of Aromatase Enzymatic Activity and Expression

Approximately 70% of all breast cancer cases are ER-positive, where ER activation by estrogen plays a key role in the stimulation of breast cancer growth and progression.[196] The use of endocrine therapy, either selective estrogen-receptor modulators (SERMs) or aromatase inhibitors (AIs), as an adjuvant treatment has demonstrated improvement in the disease-free survival of ER-positive breast cancer patients.[197] However, the risk of side effects associated with long-term AI use has triggered research into the discovery of a natural product-based new generation of AIs.[51]

Aromatase is a member of the cytochrome P450 superfamily of enzymes that catalyses the conversion of testosterone to estrogen in the final step of estrogen biosynthesis.[198] Aromatase expression is tissue-specific and dependent upon 10 alternative untranslated exons I.[199] Aromatase expression in normal breast adipose tissues is maintained at a low level, driven primarily by promoter I.4 and minimally by promoters

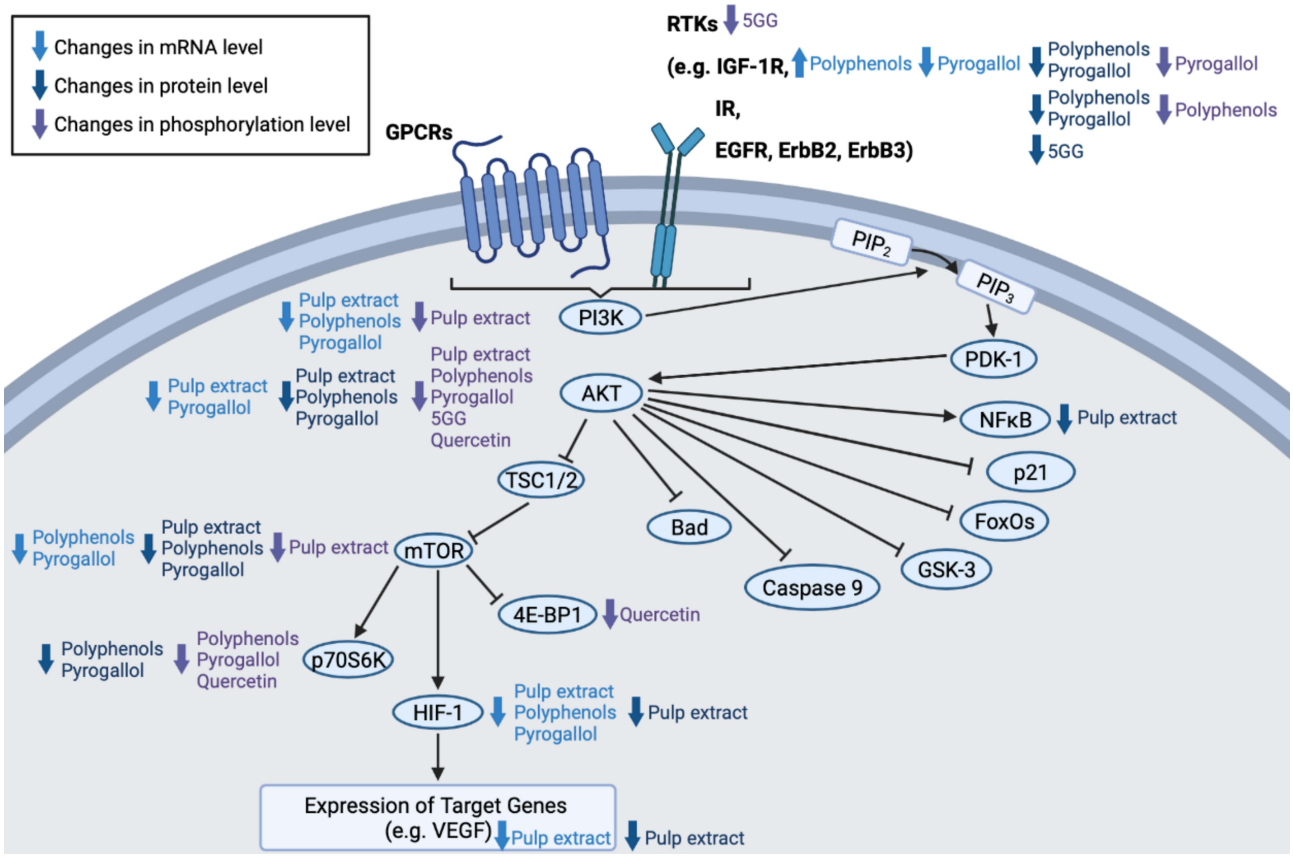


Figure 3 Suppression of PI3K/AKT/mTOR pathway by *M. indica* extracts and phytochemicals via reduction of pathway components' mRNA, total protein and phosphorylated protein levels.

Abbreviations: AKT, protein kinase B; Bad, Bcl-2-associated agonist of cell death; EGFR, epidermal growth factor receptor; ErbB2, Erb-B2 receptor tyrosine kinase 2; ErbB3, Erb-B2 receptor tyrosine kinase 3; FoxOs, mammalian forkhead transcription factors of the O class; GPCRs, G-protein-coupled receptors; GSK-3, glycogen synthase kinase-3; HIF-1, hypoxia-inducible factor-1; IGF-1R, iInsulin-like growth factor-1 receptor; IR, Insulin receptor; mTOR, mammalian target of rapamycin; NFκB, nuclear factor kappa B; PDK-1, phosphoinositide-dependent kinase-1; PIP_2, phosphatidylinositol 4, 5-bisphosphate; PIP_3, phosphatidylinositol 3, 4, 4-triphosphate; PI3K, phosphoinositide 3-kinase; p70S6K, P70 S6 kinase; RTKs, receptor tyrosine kinases; TSC1/2, tuberous sclerosis proteins 1 and 2; 4E-BP1, eukaryotic translation initiation factor 4E-binding protein 1.

I.3 and PII.[200] In estrogen-dependent breast cancer, however, elevated aromatase expression has frequently been observed to be driven by mainly promoters I.3, I.4, I.7 and PII.[200,201]

In a study, ethanolic *M. indica* peel extract was reported to dose-dependently lower the activity of human placental aromatase, with IC_{50} at 86.09 μg/mL.[51] The same study also reported that ethanolic *M. indica* peel extract (33 or 66 μg/mL) can significantly reduce aromatase mRNA level in MCF-7 cells. The extract at 33 μg/mL caused a significant reduction in I.3-containing mRNA level (78%), a significant increase in I.6-containing mRNA level (1478%), a non-significant increase in I.7- and I.f-containing mRNA levels (294% and 212%) and non-significant change in PII-containing mRNA level.[51] The extract at 66 μg/mL, on the other hand, caused a significant reduction in I.3-, I.7- and PII-containing mRNA levels (94%, 89.7% and 82%), a significant increase in I.6-containing mRNA level (500%) and a non-significant increase in I.f-containing mRNA level (100%).[51] These observations collectively suggest the potential of *M. indica* peel extract to act as a tissue-specific AI in ER-positive breast cancer.[51]

Modulation of Intracellular Calcium Signalling

The calcium (Ca^{2+}) signalling is implicated in multiple cellular processes such as autophagy,[202] cell cycle,[203] death,[204] differentiation,[205] division,[206] invasion,[207] metabolism,[208] migration[209] and transcription.[210] The key to Ca^{2+} signalling is the differential distribution of Ca^{2+} concentrations across cell membranes, between extracellular space and cytoplasm as well as between cytoplasm and organelles such as ER and Golgi apparatus.[211] Movement of Ca^{2+} ions down their electrochemical gradients produces Ca^{2+} signals.[211] This can occur with electrical, hormonal or mechanical stimulation

of cells, which can activate either GPCRs that typically trigger Ca^{2+} mobilisation from intracellular stores via lipid signal transduction; or ion channels that allow Ca^{2+} movement through central pore opening.[212] The resulting increase in cytoplasmic Ca^{2+} concentration and Ca^{2+}-binding of calcium-binding proteins (CBPs) ultimately leads to the initiation of cellular functions.[213]

The dysregulated expression of a number of Ca^{2+} channels and pumps has been reported in either breast cancer tissues or breast cancer cell lines, whereby they result in the remodelling of Ca^{2+} signalling.[214] There have been evidences linking altered Ca^{2+} signalling to various malignant functions such as cell proliferation, migration, invasion and metastasis in breast cancer.[211] Ca^{2+} channels and pumps, therefore, may be potentially useful anti-cancer targets in breast cancer treatment.

Taing et al[53] evaluated the effects of methanolic *M. indica* peel and pulp extracts (30 or 60 μg/mL) on Ca^{2+} signalling in MCF-7 cells. In untreated MCF-7 cells, adenosine triphosphate (ATP) stimulation resulted in a rapid increase in intracellular Ca^{2+} concentration $[Ca^{2+}]_I$, which was followed by a recovery to basal $[Ca^{2+}]_I$.[53] In extract-treated MCF-7 cells, however, significant reduction in peak relative $[Ca^{2+}]_I$ and delay in $[Ca^{2+}]_I$ recovery were noted.[53] Further studies are thus required to ascertain the target(s) upon which the extracts are acting to cause the observed changes in Ca^{2+} signalling, and to assess if the extracts have an influence on any Ca^{2+}-dependent signalling pathways.[53]

Studies by Chien et al[149] and Chou et al)[148] also showed that quercetin (250 or 150 μM) can elevate cytosolic Ca^{2+} level in MDA-MB-231 and MCF-7 cells, respectively. These findings suggest that further studies would be required to evaluate the influence of quercetin on Ca^{2+}-dependent signalling pathways and the link between this influence and quercetin's anti-breast cancer activity.[148,149]

Inhibition of Nuclear Factor Kappa B Signalling Pathway

Nuclear factor kappa B (NFκB), a pro-inflammatory transcription factor, is expressed in virtually all cell types.[215] The NFκB family of transcription factors comprises five members, including c-Rel, NFκB1 (p50), NFκB2 (p52), RelA (p65) and RelB.[216] Different combinations of these members make up homodimeric or heterodimeric NFκB, with the most common combination being a p65/p50 heterodimer.[216,217] NFκB dimers are usually complexed with the inhibitor of NFκB (IκB), which retains NFκB dimers in the cytoplasm and thereby prevents NFκB dimers from binding DNA.[218] NFκB activation can occur via two major signalling pathways (ie, classical/canonical and alternative/non-canonical pathways).[219] The eventual phosphorylation and ubiquitination of IκB within their signal responsive domain (SRD) results in signal responsive degradation, thereby releasing NFκB from IκB and enabling NFκB to bind to κB sites within the promoters of their target genes.[218]

Alterations of NFκB pathway are often observed in solid and hematopoietic malignancies.[220] For instance, abnormal, constitutive NFκB activation is frequently seen in breast cancer, and it is believed to contribute to breast cancer development and progression by promoting angiogenesis, cell survival, proliferation, metastasis as well as resistance to chemotherapy, endocrine therapy and radiotherapy.[216,221–225] NFκB pathway has thus been considered as a potential target for breast cancer therapy.

A study by García-Rivera et al[34] reported that Vimang (200 μg/mL), mangiferin (100 μg/mL) and gallic acid (10 μg/mL) can inhibit the classical NFκB activation pathway in MDA-MB-231 cells by inhibiting the phosphorylation of IκB kinase alpha (IKKα; at serines 176 and 180) and IκB kinase beta (IKKβ; at serines 177 and 181), which can consequently result in reduced IκB degradation, p65 nuclear translocation and NFκB DNA binding. The optimal activation of NFκB also involves the phosphorylation of its functional domains by various protein kinases.[226,227] In the same study, Vimang and gallic acid were observed to inhibit the MEK/ERK/p90RSK/MSK signalling cascade while mangiferin appeared to have insignificant effect.[34] Since gallic acid can inhibit both classical and parallel NFκB activation pathways while mangiferin can inhibit only the classical NFκB activation pathway, gallic acid is likely to be a more potent NFκB inhibitor.[34] As a consequence of NFκB pathway inhibition, there was also a significant reduction in the expression of NFκB target genes, including interleukin (IL)-6, IL-8, VEGF, Bcl-2, XIAP, COX-2 and C-X-C chemokine receptor type 4 (CXCR4), in MDA-MB-231 cells treated with Vimang, mangiferin or gallic acid.[34]

Consistent with the findings of García-Rivera et al,[34] a previous study conducted by Sarkar et al[228] also reported on the inhibitory effect of mangiferin on NFκB activation. In the study, mangiferin (0.5–10 μg/mL) suppressed TNF-stimulated activation of NFκB in MCF-7 cells. Figure 4

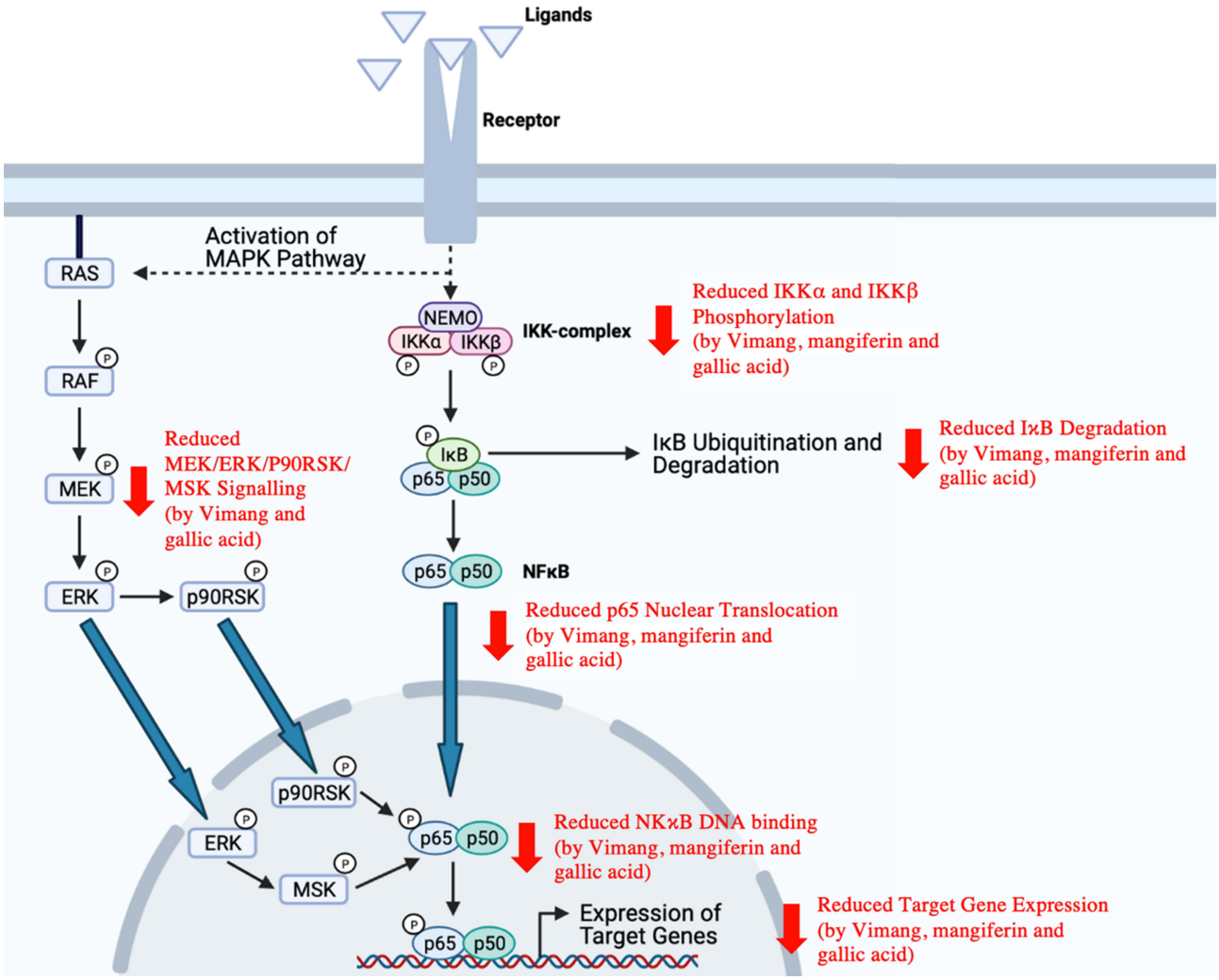


Figure 4 Inhibition of classical and parallel NFκB activation pathways by *M. indica* extracts and phytochemicals.
Abbreviations: ERK, extracellular signal-regulated kinase; IκB, inhibitor of NFκB; IKKα, IκB kinase alpha; IKKβ, IκB kinase beta; MAPK, mitogen-activated protein kinase; MSK, mitogen- and stress-activated kinase; NEMO, Nuclear factor kappa B essential modulator; p90RSK, 90 kDa ribosomal s6 kinase.

illustrates various processes along the NFκB signalling pathway that have been shown to be suppressed by *M. indica* extracts and phytochemicals.

Inactivation of β-Catenin Pathway

Wnts, a family of 19 secreted glycoproteins, are chief regulators of β-catenin activity.[229] In the absence of Wnt signal, β-catenin is constantly maintained at a low cytoplasmic level via the action of a destruction complex consisting of Axin, adenomatous polyposis coli protein (APC), casein kinase 1 (CK1) and GSK-3, where β-catenin phosphorylation by CK1 and GSK-3 eventually leads to its ubiquitination and proteasomal degradation.[230] Activation of the Wnt/β-catenin pathway is initiated with the binding of Wnt to the Frizzled (Fz) receptor and its co-receptor [ie, low-density lipoprotein receptor-related protein 5/6 (LRP5/6)].[231] The subsequent recruitment of Dishevelled (Dvl) protein, the phosphorylation and activation of LRP5/6 as well as the recruitment of destruction complex eventually ceases β-catenin degradation, thereby leading to β-catenin accumulation and nuclear translocation.[231] The expressions of Wnt target genes are usually repressed by T cell-factor/transducin-like enhancer of split (TCF/TLE) proteins and histone deacetylase (HDAC).[232] As β-catenin binds to lymphoid enhancer factor (LEF)/TCF family of transcription factors to form a binding complex, it can act as a co-activator for LEF/TCF proteins to activate the expression of Wnt target genes involved in various biological processes.[232,233]

Dysregulation of the Wnt/β-catenin signalling pathway has been associated with different diseases, including

human cancers.[234] Various studies have demonstrated that aberrant activation of the Wnt/β-catenin signalling pathway can promote angiogenesis, cell proliferation, migration, invasion and chemoresistance, thereby contributing to cancer initiation and progression.[235–237] For example, constitutively active Wnt/β-catenin pathway has been linked to metastasis in breast cancer.[234,238]

Mangiferin (12.5–50 μM) has been reported to downregulate matrix metalloproteinases (MMP)-7 and −9 and reverse epithelial-mesenchymal transition (EMT) in MDA-MB-231 and BT-549 cells, as evidenced by the observations of morphological changes (from fibroblast-like to cobblestone-like), significantly increased levels of epithelial marker (E-cadherin) and significantly decreased levels of mesenchymal markers (vimentin, snail and slug) following treatment.[66] Similar in vivo effects have been observed in MDA-MB-231 xenografts of mice subjected to mangiferin treatment (100 mg/kg/day; 5 weeks).[66] The same study also suggested that mangiferin can inhibit the β-catenin pathway by lowering the protein levels of inactive phospho-GSK3β (p-GSK3β) and active β-catenin both in vitro and in vivo. Furthermore, while β-catenin overexpression reversed the anti-proliferative, anti-migratory and anti-invasive effects of mangiferin in MDA-MB-231 and BT-549 cells, β-catenin silencing downregulated MMP-7, MMP-9 and snail, indicating that the inactivation of β-catenin pathway may contribute to the anti-cancer effects of mangiferin by modulating MMP-7, MMP-9 and EMT.[66] The overall study findings by Li et al[66] on mangiferin-mediated inactivation of β-catenin pathway are as summarised in Figure 5.

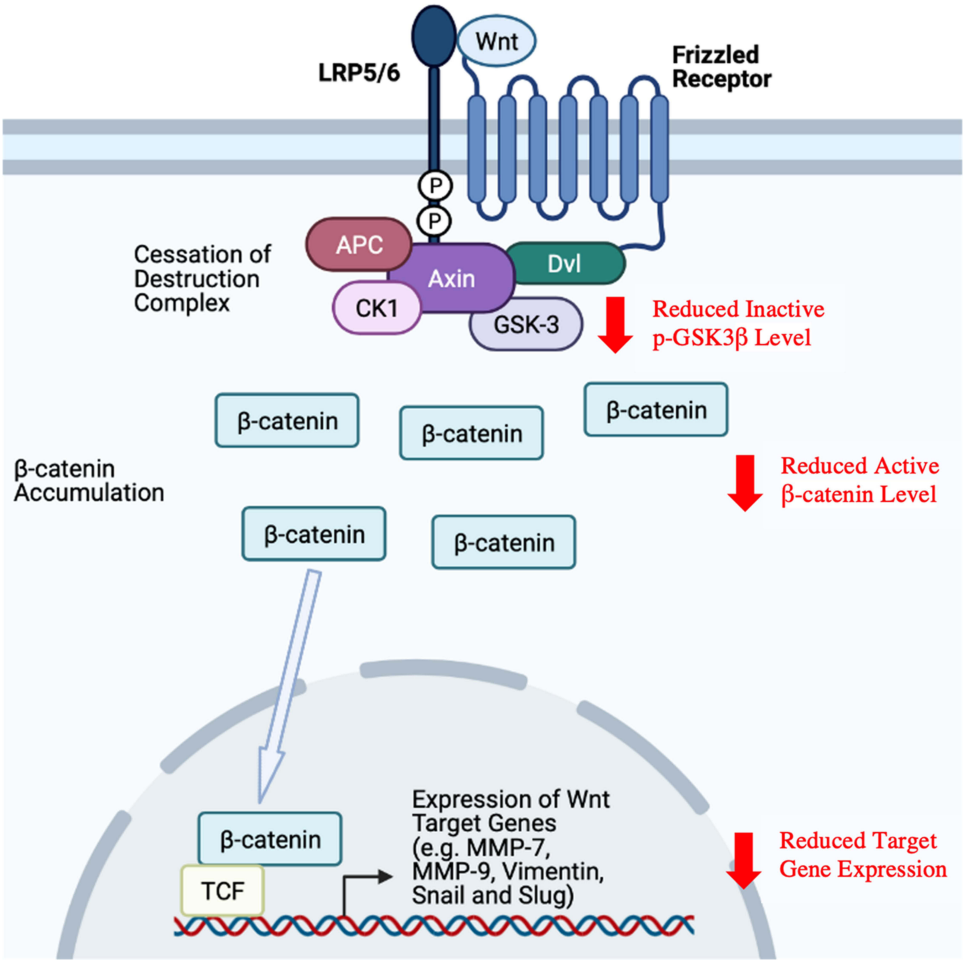


Figure 5 Inactivation of β-catenin pathway by mangiferin.
Abbreviations: APC, adenomatous polyposis coli protein; CK1, casein kinase 1; Dvl, Dishevelled; GSK-3, glycogen synthase kinase-3; LRP5/6, low-density lipoprotein receptor-related protein 5/6; MMP-7/9, matrix metalloproteinase-7/9; p-GSK3β, phospho-GSK3β; TCF, T cell-factor.

Inhibition of 3-Hydroxy-3-Methyl-Glutaryl-Coenzyme A Reductase, Proteasome and Plasmin Enzymatic Activities

3-Hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase is an enzyme that catalyses the rate-limiting step (ie, conversion of HMG-CoA to mevalonate) of cholesterol biosynthesis.[239] Low-density lipoprotein (LDL) cholesterol signalling has been associated with the promotion of cell proliferation, migration and invasion in breast cancer, suggesting the control of cholesterol level may be beneficial.[240,241] The statins, a class of cholesterol-lowering drugs, exert their hypocholesterolemic effects by blocking the activity of HMG-CoA reductase.[242] There have been evidences suggesting that the inhibition of HMG-CoA reductase by statins can impair breast cancer cell proliferation, making statins potentially useful in breast cancer treatment.[243] Cuccioloni et al [68] found that mangiferin at low μM concentrations (ie, inhibition constant of 3±0.2 μM) can similarly reduce the activity of microsomal HMG-CoA reductase. As expected, this translated to a significant dose-dependent reduction in the cytoplasmic cholesterol levels of MCF-7 and MCF-10A cells following exposure to mangiferin.[68] These effects of mangiferin on cholesterol homeostasis can, thus, potentially explain its observed anti-cancer activity in breast cancer cells.[68]

The ubiquitin-proteasome pathway (UPP) is the major pathway responsible for degrading more than 80–90% of the intracellular proteins in mammalian cells.[244] Importantly, UPP is also known to play an important role in the regulation of cellular processes involved in both tumor cell growth and survival.[245] Studies with proteasome inhibitors have demonstrated anti-proliferative and pro-apoptotic activities in tumor cells, thereby suggesting the potential usefulness of proteasome inhibitors as anti-cancer agents.[246–248] When Cuccioloni et al [68] evaluated the effect of mangiferin (1–20 μM) on various catalytic activities of isolated 20S proteasome, a significant dose-dependent reduction was observed for chymotrypsin-like (ChT-L), trypsin-like (T-L), peptidyl-glutamyl peptide hydrolase (PGPH) and branched-chain amino acid preferring (BrAAP) activities, with T-L activity being the most sensitive to inhibition by 20 μM mangiferin. When proteasome activities in the lysates of mangiferin-treated MCF-7 and MCF-10A cells were measured, significantly reduced ChT-L, T-L, PGPH and BrAAP activities were similarly observed.[68]

Plasminogen, a single-chain glycoprotein, is usually found in the blood circulation in its inactive form.[249] Upon association with cellular receptors, the resulting conformational changes induced in plasminogen promote the catalysis of its activation to plasmin by either urokinase plasminogen activator (uPA) or tissue plasminogen activator (tPA).[250] Plasmin is a serine protease that has a primary role in fibrinolysis.[251] However, the ability of plasmin to catalyse the degradation of ECM proteins and cell–cell adhesion proteins has also associated plasmin with cellular migration in both physiological (eg, embryonic angiogenesis and wound healing) and pathological (eg, tumor growth and metastasis) conditions.[252–254] This provides a rationale for plasmin inhibition in cancer management. In a study, Cuccioloni et al[68] observed that mangiferin (0–200 μM) exhibited a moderate inhibitory potency against isolated plasmin. As expected, mangiferin was also reported to cause a significant dose-dependent reduction in the cleavage of E-cadherin by plasmin in both MCF-7 and MCF-10A cells.[68] The plasmin inhibitory effect of mangiferin can thus, at least partly, help to explain its observed anti-migratory activity in breast cancer cells.[68]

Inhibition of Rac1/WAVE2 Signalling Pathway

Rho-family of small GTPases play an important role in the regulation of a range of cellular processes, including cell apoptosis, motility, mitosis and proliferation.[255] Cell division control protein 42 homolog (Cdc42) and Ras-related C3 botulinum toxin substrate 1 (Rac1), two members of the Rho-family of small GTPases, have specifically been associated with the activation of actin polymerisation and thereby, the modulation of cellular cytoskeletal reorganisation.[256] Wiskott–Aldrich Syndrome protein (WASP) and WASP-family verprolin-homologous protein (WAVE), the respective downstream effectors of Cdc42 and Rac1, are capable of activating the actin-related protein 2/3 (Arp2/3) complex that is the major cellular machinery for actin nucleation.[257] Activation of the Arp2/3 complex eventually results in the remodelling of actin filament networks as well as the formation of membrane protrusions and ruffles required for cellular motility in tumor cells.[256,258] Despite the existence of other members of the WAVE family of proteins, it has been shown that WAVE2 tends to be the most important in mediating breast cancer cell motility.[259] As such, inhibiting actin

polymerisation via the suppression of Rac1/WAVE2 signalling pathway may be effective in preventing metastasis.

It was reported by Deng et al[67] that mangiferin (2.5–10 μM) can induce a significant dose-dependent reduction in the protein levels of Rac1/Cdc42, phospho-Rac1/Cdc42 (pRac1/Cdc42), WAVE2, Arp2 and Arp3 in MDA-MB-231 cells, which is indicative of a suppression of the Rac1-WAVE2 signalling pathway (Figure 6). The authors linked this suppressive effect of mangiferin to its observed anti-migratory and anti-invasive activities in breast cancer cells.

Modulation of Estrogen Receptor Activity

ERs belong to the steroid hormone superfamily of nuclear receptors.[260] Upon activation by estrogen binding, the subsequent ER-mediated signalling can occur in either a genomic or non-genomic manner.[261] Direct genomic signalling, the classical estrogen signalling mechanism, involves conformational change and dimerization of ER, nuclear translocation of ER complex and their subsequent interaction with chromatin in estrogen response elements (EREs) to directly regulate gene expression.[262] In contrast, non-genomic signalling is mediated by membrane-bound ER and can indirectly regulate gene expression via the activation of various intracellular signalling cascades.[263]

There are two structurally and functionally distinct ER subtypes (ie, ERα and ERβ), both of which are expressed in normal breast tissues.[264] In breast cancer, while ERα expression is frequently found to be upregulated and is clearly associated with both prognosis and endocrine treatment response, ERβ expression is usually observed to be down-regulated and has no clear association with any clinical parameters.[264] ERα and ERβ have been known to play a different role in tumorigenesis, where ERα is proliferative while ERβ appears to be anti-proliferative.[265] However, it has been reported that ERβ can oppose the proliferative activity of ERα in co-expression, possibly via the formation of ERα/β heterodimers.[266] Moreover, ERα/β heterodimer-selective ligands have demonstrated growth-inhibitory effect on tumor cells and thus can potentially be useful in the treatment of ERα- and ERβ-positive breast cancer.[266]

To elucidate the mechanisms underlying the observed anti-breast cancer activities of *M. indica* phytochemicals, a study examined the effects of quercetin, mangiferin and norathyriol (1–1000 μM) on the ERE transactivation activities of ERα and ERβ in Cos-7 cells.[75] While it was reported that all three *M. indica* phytochemicals are capable of activating ERα, only norathyriol is capable of activating ERβ.[75] Therefore, further studies are required to investigate the link of these differential ER-modulatory effects to the observed lack of anti-proliferative effect of

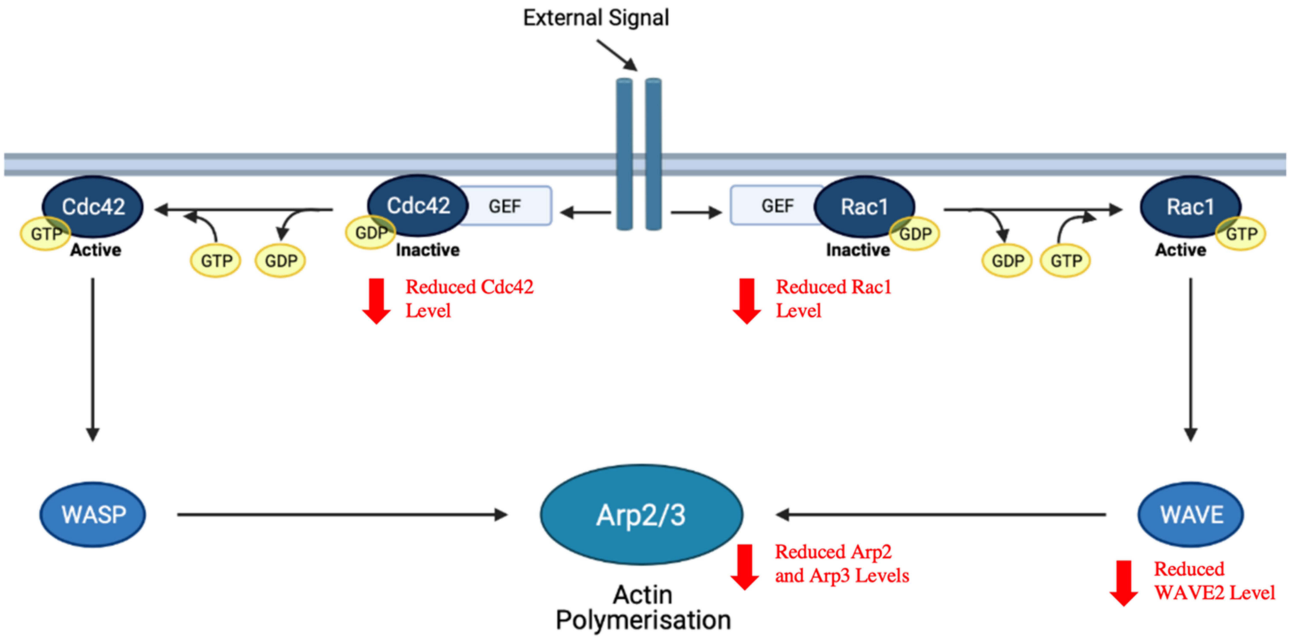


Figure 6 Inhibition of Rac1/WAVE2 signalling pathway by mangiferin. Arp2/3, activating the actin-related protein 2/3; Cdc42, cell division control protein 42 homolog; GEF, guanine nucleotide exchange factor; Rac1, Ras-related C3 botulinum toxin substrate 1; WASP, Wiskott–Aldrich syndrome protein; WAVE, WASP-family verprolin-homologous protein.

mangiferin as well as the observed anti-proliferative effects of quercetin and norathyriol on MCF-7 cells; and to evaluate the effects of these *M. indica* phytochemicals on ERα/β heterodimer activity.[75]

5GG, another *M. indica* phytochemical, has also been reported to exert growth-inhibitory effect on MCF-7 by inhibiting ERα function.[94] Specifically, the study revealed that 5GG (1–40 μM) can inhibit ERα activity by promoting ERα degradation through the lysosomal pathway or blocking all known pathways of ERα activation – estrogen-mediated activation, growth factor-induced phosphorylation of the amino-terminal activation function-1 (AF-1) and cyclin D1-mediated activation.[94]

Activation of AMP-Activated Protein Kinase Signalling Pathway

AMP-activated protein kinase (AMPK) is a serine/threonine kinase complex consisting of a catalytic subunit (AMPK-α) and two regulatory subunits (AMPK-β and AMPK-γ).[267] It is regarded as a sensor of intracellular adenosine nucleotide levels, where it is activated in response to cellular stresses that can reduce intracellular ATP level.[268] AMPK activation involves the phosphorylation of its conserved threonine 172 residue by an upstream kinase such as Ca^{2+}/calmodulin-dependent protein kinase β (CaMKKβ), liver kinase B1 (LKB1) and transforming growth factor β activated kinase 1 (TAK1).[269] More recent studies have further suggested AMPK's role as an oxidative stress sensor in addition to its traditional role as an energy sensor, where it can be activated in response to increased intracellular ROS/reactive nitrogen species (RNS) levels.[270–272]

AMPK has physiological roles in the regulation of cell growth, metabolism, polarity and autophagy, and AMPK activation has been recognised to exert beneficial effects in various pathological conditions.[268] The downregulation of AMPK has been associated with poor outcomes in breast cancer patients.[273] However, there have been reports that suggest AMPK activation can exert a growth-inhibitory effect on breast cancer cells, thus making AMPK signalling pathway a promising target in breast cancer treatment.[274–276]

A study proposed that the observed inhibitory effects of *M. indica* polyphenols (0.8 mg GAE/day; 4 weeks) and pyrogallol (0.2 mg/day; 4 weeks) on MCF10DCIS xenograft tumor growth can be attributed at least partly to AMPK pathway activation and AKT/mTOR pathway suppression.[57] In the study, mice of both treatment groups showed elevated levels of Sestrin2, phosphorylated AMPK and Beclin1 as well as reduced levels of mTOR and pmTOR. However, while elevated level of LKB1 was only seen in pyrogallol-treated mice, elevated levels of Unc-51-like kinase (ULK) and phosphorylated ULK were only seen in polyphenol-treated mice.[57] Sestrin2, a highly conserved stress-inducible protein, is known to be capable of activating AMPK that can in turn inhibit mTOR.[277] In addition, both ULK and Beclin1, which are involved in the induction of autophagic process, are also the downstream targets of AMPK.[278] Subsequent in vitro assays performed on MCF10DCIS cells linked AMPK pathway activation induced by *M. indica* polyphenols and pyrogallol (1–20 mg/L) to their abilities to increase ROS generation.[57] Moreover, it was reported that pyrogallol can also activate the AMPK pathway by binding directly to the allosteric site of AMPK or upregulating AMPK expression.[57]

On the other hand, quercetin's anti-cancer activity has been linked to an upregulation of AMPK signalling in another study, whereby quercetin (15 μM) was observed to significantly increase AMPK phosphorylation (by two-fold) in MDA-MB-231 cells.[150] Figure 7 summarises the study observations suggesting an upregulation of the AMPK signalling pathway following treatment with *M. indica* extracts and their phytochemicals.

Modulation of Cell Cycle Regulators

Cell cycle is a process comprising four major phases (ie, G1-, S-, G2- and M-phases), during which the doubling of cellular components and their accurate segregation into two daughter cells occur.[279] Transition through each phase of the cell cycle is under tight regulation by specific proteins.[279] Cyclin-dependent kinases (CDKs), a family of serine/threonine protein kinases, are the key regulatory proteins that control cell cycle progression when complexed with their regulatory subunits (ie, cyclins).[280] G1-to-S-phase transition involves the activation of CDK4, 6 activity by D-type cyclin binding.[281] One target of cyclin D-CDK4, 6 activity is the retinoblastoma tumor suppressor protein (Rb), which will dissociate from E2F transcription factors following phosphorylation to enable E2F-induced expression of cyclins E, A and thymidylate synthase, etc.[282,283] Continued phosphorylation and complete inactivation of Rb in the late G1-phase is achieved by the complexes formed from cyclins E and A as well as CDK1 and 2.[284] On the other hand, cyclinB-CDK1 activity is known to drive cellular events associated with mitosis, thereby facilitating G2-to-M-phase transition.[285] The activity of cyclin-CDK complexes can be counteracted by two gene families of CDK inhibitors (CKIs), thereby resulting in cell cycle arrest.[286] While INK4 gene family members (p16INK4a, p15INK4b, p18INK4c and

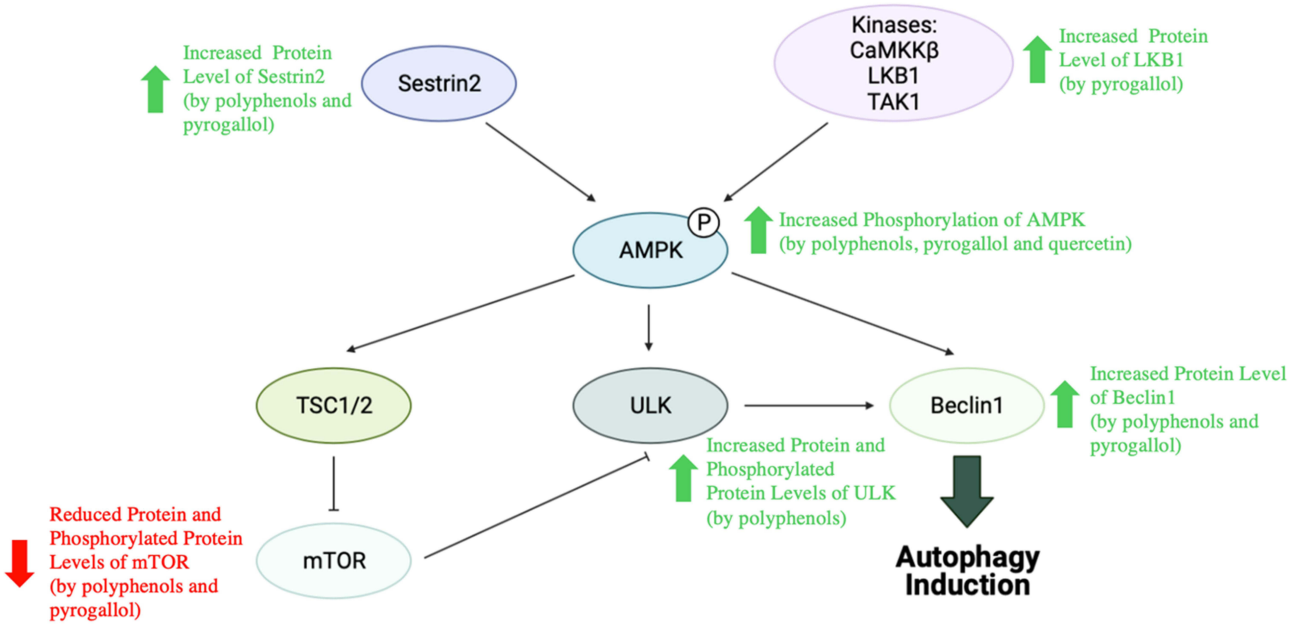


Figure 7 Activation of AMPK signalling pathway by *M. indica* extracts and phytochemicals.
Abbreviations: AMPK, AMP-activated protein kinase; CAMKKβ, calcium/calmodulin-dependent protein kinase β; LKB1, liver kinase B1; mTOR, mammalian target of rapamycin; TAK1, transforming growth factor β activated kinase 1; TSC1/2, tuberous sclerosis proteins 1 and 2; ULK, Unc-51-like kinase.

p19INK4d) are capable of inhibiting CDK4, 6 activity, Cip/Kip gene family members (p21Cip1/Waf1/Sdi1, p27Kip1 and p57Kip2) can interfere with the activities of cyclins D-, E-, and A-CDK complexes.[286]

Deregulation of cell cycle, which confers cells with the ability to undergo cell division endlessly, is one of the hallmarks of cancer.[287] In breast cancer, deregulation of cell cycle often results from an aberrant activation of CDKs and cyclins as well as an inactivation of tumor suppressors.[288] As such, several CDK4/6 inhibitors (ie, abemaciclib, palbociclib and ribociclib) have been approved by FDA for the treatment of breast cancer, and a number of cell cycle inhibitors (eg, TTK protein kinase [TTK] and polo-like kinase 4 [PLK4] inhibitors) are currently under development.[287]

Chen et al[95] reported the ability of 5GG (50 μM) to induce a G1-phase arrest in MCF-7 cells and proceeded to investigate the underlying mechanisms of this arrest. Overall study findings suggested that 5GG treatment can increase the protein level of p27Kip, which can in turn bind to and inhibit CDK2 and 4 kinase activities, resulting in a reduced Rb phosphorylation and thus preventing E2F-induced expression of S-phase genes.[95] Moreover, it was also reported that 5GG may block the association of p21Cip with cyclin D-CDK4 complexes, which would usually be required to increase the stability and activity of cyclin D-CDK4 complexes implicated in G1-to-S-phase transition.[95]

Gallic acid (2–12 μg/mL) has also been reported to induce S- and G2/M-phase arrest in MCF-7 cells, which is linked to its ability to dose-dependently lower the protein levels of cyclin A, cyclin B1, cdc2/CDK1 and CDK2.[111] Additionally, gallic acid can also dose- and time-dependently downregulate S-phase kinase-associated protein 2 (Skp2) and prevent it from ubiquitinating p21Cip1 and p27Kip1, thereby preventing the proteasomal degradation of p21Cip1 and p27Kip1 and leading to cell cycle arrest.-[111] However, as the silencing of p27Kip1 but not p21Cip1 can significantly diminish gallic acid-induced increase in G2/M-phase ratio, p27Kip1 may play a more important role than p21Cip1 in the observed cell cycle arrest induced by gallic acid.[111]

Quercetin can also induce cell cycle arrest in breast cancer cell lines. For instance, Chien et al[149] observed a G2/M-phase arrest in MDA-MB-231 cells following quercetin treatment (200 μM), and correlated this with a reduction in the protein levels of cyclin A, cyclin B and thymidylate synthase as well as an increase in the protein levels of p53 and p57. On the other hand, Chou et al[148] reported that quercetin (150 μM) can induce a S-phase arrest in MCF-7 cells by downregulating the protein levels of CDK2, cyclin A, cyclin E and thymidylate synthase as well as upregulating the protein levels of p53 and p57.

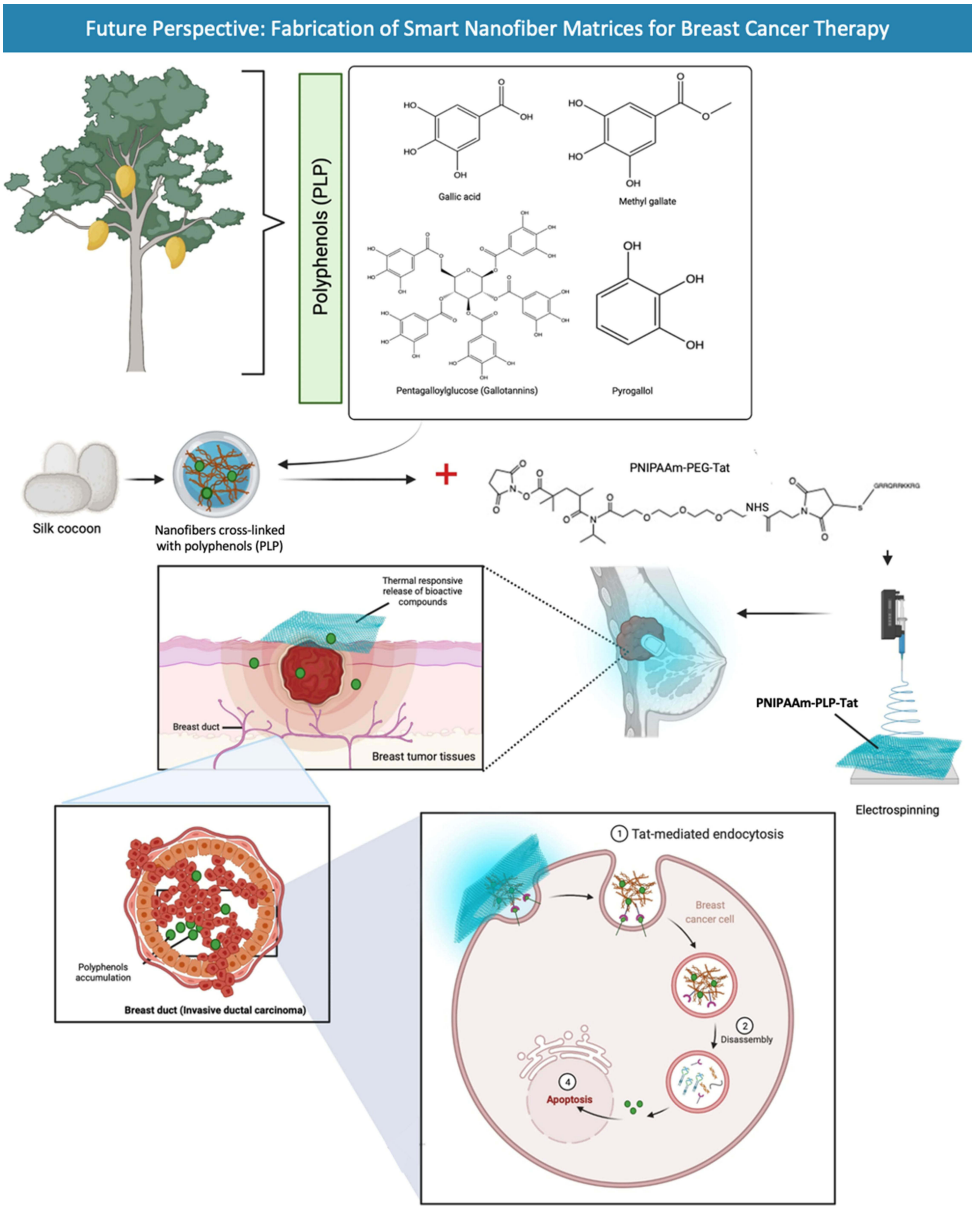


Figure 8 Fabrication of smart nanofiber matrices for breast cancer therapy. Polyphenolic compounds (PLP) from *M. indica* have been studied extensively as anti-inflammatory and anti-cancer agents. In the proposed future study, *M. indica* PLP will be cross-linked to silk fibroin (cocoon) nanofibers before being conjugated with PNIPAAm-PEG-Tat to yield PNIPAAm-PLP-Tat, which can then be released in a thermal responsive burst at breast tumor tissues. The use of cell-penetrating peptide (Tat) permits targeted intracellular transport of PLP into breast cancer cells.

Conclusion and Future Perspectives

This review provides a comprehensive summary of the studies on the anti-cancer effects of extracts prepared from various parts of *M. indica* (ie, bark, kernel, leaves, peels and pulps) and its polyphenolic phytochemicals (ie, mangiferin, norathyriol, gallotannins, gallic acid, pyrogallol, methyl gallate and quercetin) in preclinical models of breast cancer. Overall, there was a reduction in cell viability, proliferation, growth, migration and invasion as well as an induction of apoptosis and cell cycle arrest. Reduced tumor weight and volume were also observed. A number of mechanisms have been proposed so far: activation of oxidative stress-induced cell death, antioxidant activity, modulation of PPARs, suppression of P13K/AKT pathway, induction of miR-126 expression, inhibition of aromatase enzymatic activity and expression, modulation of intracellular Ca^{2+} signalling, inhibition of NFκB signalling pathway, inactivation of β-catenin pathway, inhibition of HMG-CoA reductase, proteasome and plasmin enzymatic activities, inhibition

of Rac1/WAVE2 signalling pathway, modulation of ER activity, activation of AMPK signalling pathway as well as modulation of cell cycle regulators.

Further selective compound studies should be performed on *M. indica* extracts that possess anti-breast cancer activities to identify and validate phytochemicals that are responsible for the observed bioactivities. Extracts of different *M. indica* parts showed different bioactivities, suggesting variabilities in the type and amount of phytochemicals and thus highlighting the importance of targeting certain plant parts. Additionally, variabilities in bioactivities have also been observed for *M. indica* extracts despite utilisation of the same plant parts, indicating the presence of different phytochemical profiles in these extracts and further emphasizing the importance of selecting the right *M. indica* varieties, extraction method and extraction solvent. Once identified and validated, the phytochemical(s) of interest should be isolated for subsequent studies on their efficacies, toxicities, mechanisms of action and drug metabolism and pharmacokinetics (DMPK) profiles. It has been supported by various studies that multiple *M. indica* phytochemicals may act synergistically such that their combined anti-breast cancer efficacy is higher than that of a single phytochemical, suggesting the potential benefits of their combined usage in cancer treatment. Furthermore, while *M. indica* fruit consumption may be beneficial for women's health in relation to breast cancer given the reported anti-breast cancer activities of *M. indica* pulp extracts, further bioavailability studies are required to determine whether the bioactive compounds within the fruits exhibit reasonable bioavailability to exert their anti-cancer effects following fruit consumption. More committed studies, particularly clinical trials, are needed to explore the anti-breast cancer potential of *M. indica* extracts and their phytochemicals in order to validate their utility in the development of unique and potent therapeutic agents to treat breast cancer. Apart from that, the phytochemicals that are present in *M. indica* extracts may vary in concentration depending on the food source and season, hence the development of health supplements is a better option for consistent deliveries. Furthermore, chemotherapeutic agents lack tumor selectivity such that they may damage normal cells and cause major side effects. Therefore, targeted delivery of bioactive compounds to tumors is required to minimise toxicity to normal cells. Drug delivery techniques based on nanofibers have recently emerged as a potential platform for delivering bioactive compounds to tumor sites. Nanofibers cross-linked with polyphenols from *M. indica* can be coupled to PNIPAAm-PEG-Tat to enable smart tumor microenvironment-responsive targeted distribution of *M. indica* polyphenols. Because of their ability to deliver medicines and genes intracellularly, arginine-rich cell-penetrating peptides (CPPs) like transactivating transcriptional activator (TAT) have attracted a lot of attention (Figure 8). On the other hand, in-depth study into alternative delivery systems should be examined as a possibility for optimizing localisation at tumor target areas and therefore boosting therapeutic efficacy.

Consent for Publication

The final version of the manuscript was reviewed by all the authors who consented to its submission.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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