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Review Article

MORINGA OLEIFERA: AN INTEGRATED REVIEW OF PHYTOCHEMISTRY, MOLECULAR MECHANISMS, CLINICAL TRANSLATION, AND NANO-DELIVERY SYSTEMS

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Abstract:

Moringa oleifera Lam. The “Miracle Tree” (Moraceae), a rich source of bioactive phytochemicals, has significant medicinal properties [1, 2]. The plant is utilized in traditional medicine for its various sections and modern ethnopharmacology has been trying to elucidate the exact molecular targets, therapeutic benefits and the translation potential of the plant [3]. This review brings together up to date information regarding *M. oleifera*, its major phytoconstituents such as glucosinolates, isothiocyanate (moringin), flavonoids (quercetin, kaempferol), chlorogenic acid and alkaloids [11, 12, 18]. We map out its primary molecular signaling pathways, highlighting how it regulates the Nrf2/ARE antioxidant defense, suppresses NF- κ B/MAPK inflammatory cascades, activates AMPK/GLUT4 metabolic pathways, and modulates Bcl-2/Bax/Caspase-3 apoptotic signaling [22, 23, 27]. We also discuss human clinical studies in the metabolic, immunomodulatory and antioxidant fields, where major translational gaps exist [4, 5, 31]. Finally, recent breakthroughs in biogenic green nanotechnology and smart nano-delivery systems to address native solubility and bioavailability restrictions are highlighted [44, 46]. This review integrates a framework to fill the space between benchtop phytochemistry and clinical applications [8, 9, 10].

Keywords: *Moringa oleifera*, Moringin, Phytoconstituents, Nrf2 Pathway, NF- κ B Pathway, AMPK, Nano-formulations, Clinical Trials, Pharmacokinetic.

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1. INTRODUCTION:

Moringa oleifera Lam. is a native tree of South Asia and belongs to the family Moringaceae [1, 6]. It has been documented in Ayurveda in the past such as Charaka Samhita and used in traditional ethnomedicine in Africa and Asia. It has become one of the main subjects of ethnopharmacological studies in recent years [19]. Almost all the anatomical sections of the plant including leaves, seeds, pods, flowers, bark, and roots have pharmacological activity including antioxidant, anti-inflammatory, antidiabetic, neuroprotective, cardioprotective, hepatoprotective, and antineoplastic properties [2, 19, 20]. The tree is very hardy, growing in dry and semi-arid climates and is a valuable nutritional and therapeutic resource in underdeveloped countries [40].

Its bioactivity has been extensively validated in preclinical studies [21-25], but clinical translation is hampered by substantial inter-batch variability, poor

oral bioavailability of important polyphenols and a lack of large-scale, placebo-controlled clinical trials [35, 39]. In view of these challenges, the present comprehensive review aims to systematically map the phytochemical landscape of *M. oleifera*, directly correlate these compounds with intracellular signaling networks, critically assess recent human clinical evidence and explore modern nano-formulation paradigms that can improve its therapeutic index [46, 47].

2. PHYTOCHEMICAL ARCHITECTURE AND BIOACTIVE PROFILING

One of the reasons why *M. oleifera* is so effective as a medicinal agent is that it contains a significant amount of both primary and secondary metabolites. Isothiocyanates, flavonoids, and phenolic acids are primary pharmacophores that are accountable for anti-inflammatory, cytotoxic, and chemopreventive properties of this substance [11, 12, 14, 18].

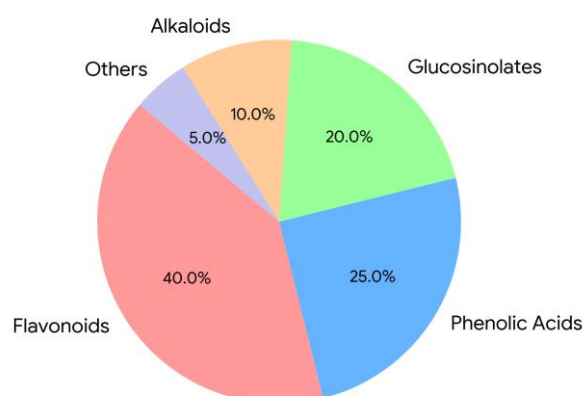


Figure 1: Relative distribution of major secondary metabolites within *M. oleifera* leaf extracts.

2.1 Glucosinolates and Isothiocyanates

Cruciferous vegetables are rich in aliphatic glucosinolates, but *M. oleifera* contains uncommon aromatic glucosinolates, particularly glucomoringin [12]. Glucomoringin is hydrolyzed by endogenous myrosinase or digestive microbiota enzymes to generate the active isothiocyanate: moringin [13]. Moringin is a powerful inducer of phase II cytoprotective enzymes and has important chemopreventive properties [13, 25].

2.2 Polyphenols and Flavonoids

Leaf tissues include large levels of flavonol glycosides, namely quercetin-3-O-glucoside and kaempferol-3-O-glucoside, rutin and chlorogenic acid [15, 16, 17]. Quercetin acts as a direct scavenger of ROS, chelates transition metals and regulates membrane lipid peroxidation while kaempferol is highly involved in metabolic regulation thru activation of AMPK [27, 28].

Table 1: Comprehensive Profiling of Major Phytoconstituents in *Moringa oleifera*

Class	Compound Name	Primary Source	Target Receptors/Pathways	Biological Activity
Isothiocyanates	Moringin (MIC-1)	Leaves, Seeds	Keap1, NF-κB p65, Nrf2	Antioxidant, Anti-inflammatory [22]
Flavonoids	Quercetin	Leaves, Flowers	TNF-α, IL-6, MAPK/ERK	Cardioprotective, Anti-apoptotic [17]

Class	Compound Name	Primary Source	Target Receptors/Pathways	Biological Activity
Flavonoids	Kaempferol	Leaves	AMPK, Akt/mTOR, GLUT4	Anti-diabetic, Anti-obesity [27]
Phenolic Acids	Chlorogenic Acid	Leaves, Pods	α -Glucosidase, G6Pase	Antihyperglycemic, Hepatoprotective [28]
Carbamates	Niazimicin	Seeds, Bark	Ca ²⁺ channels, EBV activation	Hypotensive, Anti-tumor promoter [18]
Sterols	β -Sitosterol	Seeds, Roots	HMG-CoA Reductase, PPAR- γ	Hypolipidemic, Anti-atherosclerotic [21]
Alkaloids	Moringine / Moringinine	Bark, Roots	Acetylcholinesterase, Monoamines	Neuroprotective, Anticonvulsant [54]

3. INTEGRATED MOLECULAR MECHANISMS OF ACTION

Understanding the specific intracellular signaling networks modulated by *M. oleifera* bioactives is critical for rational drug design and targeted therapeutics.

3.1 The Nrf2/ARE Antioxidant Pathway

M. oleifera is responsible for the generation of an endogenous antioxidant response, which is mediated by the activation of the Nrf2 pathway [22, 25]. This is the principal mechanism by which antioxidant response is mediated. When the physiological conditions are suitable, the protein known as Keap1, which is responsible for mediating the proteasomal destruction of Nrf2, is also responsible for maintaining it in the cytoplasm. Moringin and quercetin are known to undergo electrophilic modification of the crucial cysteine residues on Keap1, which is a phenomena that has been widely documented [22]. A modification of Cys151 is an example of this kind of change. The Keap1-Nrf2 complex is destabilized as a consequence of this alteration, which also makes it feasible for Nrf2 to translocate in nucleus, heterodimerize with sMaf, and bind to the ARE. The transcription of essential phase II detoxifying and anti-oxidant enzymes such as HO-1, NQO1, SOD, and GSH-Px is a result of this process [22, 26, 30].

3.2 Modulation of NF- κ B and MAPK Inflammatory Cascades

inhibit intestinal α -glucosidase and α -amylase and decrease postprandial hyperglycaemia peaks [29].

The existence of chronic inflammation is the underlying cause of many non-contagious metabolic and degenerative diseases. Extracts of *M. oleifera* have been demonstrated to suppress inflammatory signals via the NF- κ B and MAPK pathways [1, 23, 24]. Bioactive compounds are able to suppress phosphorylation and subsequent degradation of I κ B α , an inhibitor of NF- κ B [23]. In this manner, the NF- κ B p50/p65 heterodimer is always found in an inactive state in the cytoplasm. Inhibition of NF- κ B nuclear translocation may lead to reduced expression of pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6, and production of important inflammatory enzymes including iNOS and COX-2 [1, 23]. Polyphenols inhibit the activation of JNK and p38 MAPK resulting in a decreased oxidative tissue damage and neutrophil production [24].

3.3 AMPK Activation and Glucose Homeostasis

The kaempferol and chlorogenic acid of *M. oleifera* phosphorylate AMP-activated protein kinase at Thr172 in metabolic tissue (skeletal muscle, liver and adipose) [27, 28]. Activation of AMPK promotes peripheral glucose uptake by increasing the translocation of vesicles containing GLUT4 to plasma membrane, independent of insulin signalling [28]. AMPK activation in the liver reduces hepatic glucose production thru the suppression of key gluconeogenic genes (PEPCK and G6Pase) [27]. *M. oleifera* polyphenols

Table 2: Key Intracellular Signaling Cascades Modulated by *M. oleifera*

Pathway	Primary Modulator	Upstream Effect	Downstream Transcription/Proteins	Physiological Outcome
Nrf2/ARE	Moringin	Keap1 Inhibition	HO-1, NQO1, SOD, Catalase ↑	Cytoprotection, Antioxidant
NF-κB	Quercetin	IκBα stabilization	TNF-α, IL-6, COX-2, iNOS ↓	Anti-inflammatory, Immunomodulatory
AMPK	Kaempferol	Thr172 Phosphorylation	GLUT4 translocation, PEPCK ↓	Enhanced Insulin Sensitivity
mTOR/Akt	Rutin	Akt inhibition	Bcl-2 ↓, Bax ↑, Caspase-3 ↑	Apoptosis in malignant cells

4. TRANSLATIONAL CLINICAL EVIDENCE IN HUMANS: TRIALS

Although preclinical in vitro and in vivo models offer robust molecular understanding, comprehensive human trials are necessary to prove

actual clinical efficacy. Trials are necessary to prove actual clinical efficacy. Recent studies on randomized controlled trials (RCTs) show that *M. oleifera* has promise for use as an adjunctive treatment in early-stage immunological and metabolic regulation disorders [4, 5, 7, 35].

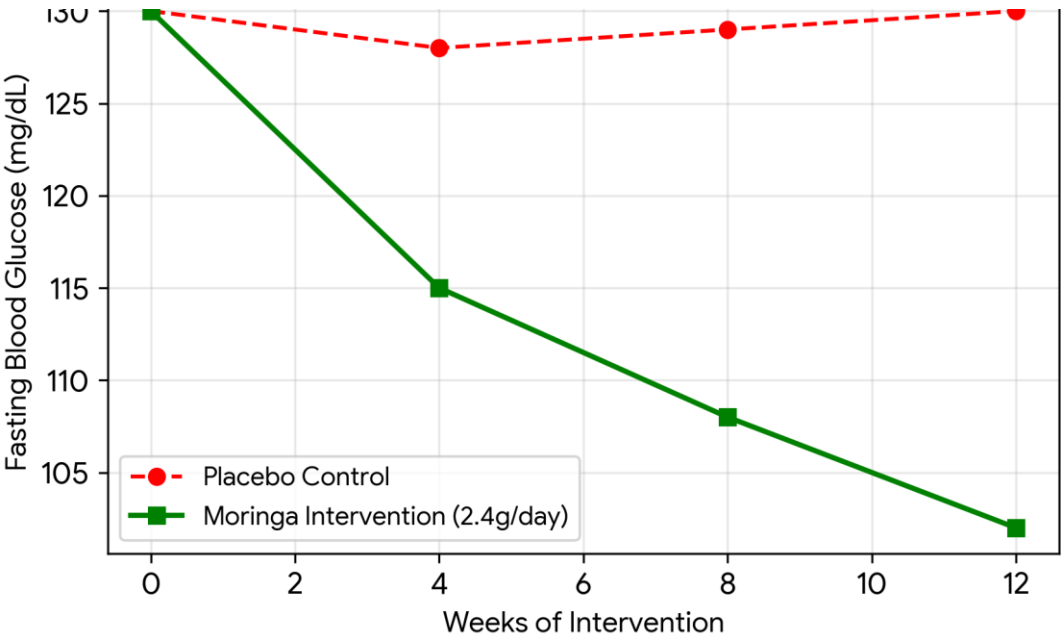


Figure 2 (derived from Gomez-Martinez et al. [4]) shows the progressive effect of *M. oleifera* leaf powder on fasting blood glucose levels in prediabetic patients over a 12-week intervention period.

4.1 The Clinical Translation Gap: Early vs. Late Intervention

Meta-analyses of clinical trials show that *M. oleifera* leaf supplementation leads to more significant and pronounced effects in prediabetic/dysmetabolic individuals than in patients with advanced, chronic

T2DM [35, 36]. HbA1c levels and fasting glucose are significantly lowered by early treatment (e.g. 2.4 to 4 g/day for 12 weeks) due to enhanced activity of β-cells and insulin sensitivity [4, 36]. However, short-term interventions (for 4 weeks or less) are not always successful in achieving full structural pancreatic depletion in well-established T2DM [7].

Table 3: Extended Summary of Human Clinical Trials Investigating *M. oleifera*

Study Population	Intervention/Dose	Duration	Biomarkers Analyzed	Clinical Findings / Outcomes	Ref
Prediabetic Adults (n=65)	Leaf powder (2.4 g/day)	12 weeks	FBG, HbA1c, TNF- α , IL-6	Fasting glucose -5.6 mg/dL; HbA1c -0.3% ; TNF- α $\downarrow$	[4]
Saharawi Adults (n=17)	Leaf powder (20 g single)	Acute	Postprandial Glucose	Reduced postprandial glucose AUC by 21%	[6]
T2DM Patients (n=60)	Leaf capsules (8 g/day)	4 weeks	FBG, HbA1c, Lipids	Minimal HbA1c change; postprandial spikes attenuated	[7]
Hyperlipidemic (n=50)	Leaf extract (1.5 g/day)	8 weeks	TC, TG, LDL-C, HDL-C	Total Cholesterol $\downarrow$ 11.2%; LDL-C $\downarrow$ 14.3%	[31]
HIV+ Adults (n=40)	Leaf powder (5 g/day)	6 months	CD4+ count, BMI, MDA	CD4+ T-cells $\uparrow$; BMI improved; Oxidative stress $\downarrow$	[32]
Hypertensive (n=45)	Leaf extract (3 g/day)	4 weeks	Systolic/Diastolic BP	Significant reduction in systolic pressure ($p < 0.05$)	[33]
Anemic Women (n=60)	Leaf powder (100 g/week)	3 months	Hemoglobin, Ferritin	Hemoglobin levels increased by 1.2 g/dL average	[37]

5. NOVEL PARADIGM SHIFT: NANOTECHNOLOGY AND SMART DELIVERY SYSTEMS

The key biopharmaceutical challenges of native *M. oleifera* bioactives include extreme hydrophobicity

(e.g. quercetin), rapid hepatic first-pass metabolism, sensitivity to gastrointestinal pH and enzymatic degradation [44, 46]. Researchers are racing to utilize *M. oleifera* extracts with new nanocarrier technologies to overcome these structural issues and increase targeted delivery [8, 10, 46].

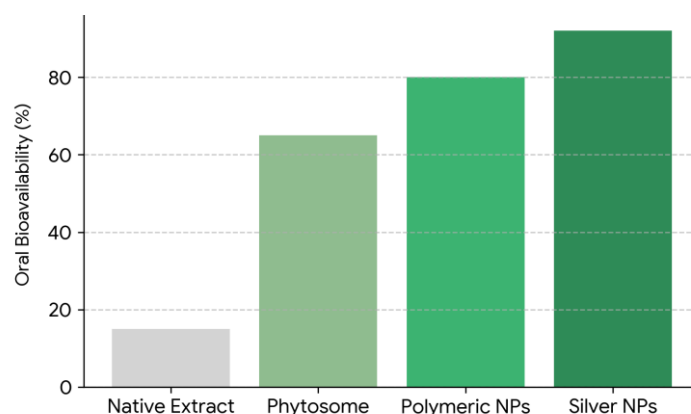


Fig. 3: The improvement of theoretical oral bioavailability by switching from native extracts to more sophisticated nanocarrier systems such as phytosomes and metallic nanoparticles.

5.1 Biogenic Synthesis of Metallic Nanoparticles

Aqueous leaf and seed extracts of *M. oleifera* are effective and eco-friendly dual reducing and capping agents in biogenic synthesis of metal nanoparticles (e.g. AgNPs, AuNPs, ZnONPs, TiO₂-NPs) [41, 42, 43, 50]. Additionally, the plant has a

high amount of polyphenols and flavonoids, which effectively decrease metal ions (e.g. Ag⁺ $\rightarrow$ Ag⁰; Au³⁺ $\rightarrow$ Au⁰) [41, 48] and form a self-assembled organic stabilizing layer for preventing agglomeration of the nanoparticles. The Au-NPs generated with the use of *M. oleifera* can produce ROS and inhibit the mitochondrial pathway, leading

to a highly selective lethal effect on A549 lung carcinoma and MCF-7 breast carcinoma cell lines exhibiting IC₅₀ values about 50 µg/mL [42]. Silver nanoparticles (AgNPs) synthesized by green route are known to have good antibacterial activity against several drug resistant pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* by damaging the integrity of the cell walls [41, 48].

5.2 Phytosomes and Polymeric Nano-Formulations

Sensitive bioactives like moringin and quercetin are shielded from enzymatic breakage in the hostile gastrointestinal environment by phospholipid-based phytosome complexes and biodegradable polymeric nanocarriers (such as PLGA and chitosan) [44, 45, 47]. When compared to unencapsulated extracts, these lipid-compatible structures improve passive transcellular intestinal absorption, increasing oral bioavailability by up to four or six times [44]. The plasma levels of *M. oleifera* extract released from PLGA nanoparticles are sustained for 72 h, and the rate of released doses is reduced [45, 47].

Table 4: Next-Generation Nanocarrier Systems for *M. oleifera* Delivery

Formulation Type	Avg. Particle Size	Bioactive / Payload	Therapeutic Application	Key Advantage	Ref
Biogenic Silver (AgNPs)	15–30 nm	Phenolics, Flavonoids	Antimicrobial, Wound healing	High surface area, biofilm disruption	[41,48]
Biogenic Gold (AuNPs)	20–45 nm	Moringin, Quercetin	Oncology (MCF-7, A549)	Targeted ROS induction, low healthy-cell toxicity	[42]
PLGA Nanoparticles	120–160 nm	Total Leaf Extract	Sustained drug delivery	Controlled release over 72h, bypass first-pass	[45]
Phytosome Complexes	150–220 nm	Quercetin, Phenolics	Hepatoprotection, Anti-diabetic	Massively enhanced intestinal membrane permeability	[44]
Zinc Oxide (ZnONPs)	40–60 nm	Aqueous Extract	Photocatalysis, Anti-inflammatory	Biocompatible, synergistic anti-inflammatory action	[43]

6. SAFETY, TOXICOLOGY, AND REGULATORY CONSIDERATIONS

Although pharmaceutical use is common, and it is a functional food, strict toxicological profile is required for pharmaceutical standardization [51, 52]. The results of the preclinical toxicological analysis revealed that the safety margin of *M. oleifera* leaves is very wide, consistent across all of them [53]. In mice models, the acute oral median lethal dose (LD₅₀) ≥ 5,000 mg/kg body weight for both aqueous and ethanolic extracts of the leaves indicating that they are virtually non-toxic when administered under acute exposure paradigms [51, 52]. Hematological, hepatic, and renal histopathology do not significantly change at therapeutic levels, according to subacute and chronic toxicity studies (varying from 30 to 90 days) [51, 55]

However, in root/bark preparations, much more care is required [54]. However, some parts having higher concentration of alkaloids such as spirachin and

moringinine. When consumed in large amounts these substances could lead to cardiac toxicity, uterine contraction, or neuromuscular obstructions [54]. Therefore, it is generally not recommended using the root extracts during pregnancy. Human intervention trials with standardized leaf powder show excellent tolerability with no report of serious adverse events (SAEs) for all dosages between 2.4 g/day and 8 g/day [4, 7, 53].

7. FUTURE DIRECTIONS AND CRITICAL TRANSLATIONAL GAPS

The following are studies that are needed to make *M. oleifera* a standardized pharmaceutical agent for future study:

1. Analytical Standardization – formulations of the extracts should be standardized by analytical means by measuring marker molecules with HPLC and using the minimum percentage of them (for example moringin or some of the quercetin glycosides) [15, 40]

2. Pharmacokinetic Mapping: In order to investigate the ADME properties of the important glucosinolates and their conversion to active isothiocyanate, extensive human studies are performed [13].

3. Microbiome-Extract Crosstalk: Insights into Individual Difference in Gut Microbiome Composition and its Implications on the Enzymatic Conversion of Glucomoringin to Moringin, a Key Factor for the Systemic Efficiency [12].

4. Phase III Clinical Validation: RCTs that are large, multi-center, placebo-controlled and specifically target subpopulations including patients requiring adjuvant immunomodulation, those with prediabetes and patients who have non-alcoholic fatty liver disease (NAFLD) [35, 39]

8. CONCLUSION:

Moringa oleifera, a multi-target botanical candidate has been scientifically proven to have great potential for functional nutraceuticals and drug development [1, 2, 3]. It contains several bioactive phytoconstituents which have interlinked molecular networks with each other and play a synergistic role in their activity, such as moringin and some flavonols. AMPK metabolic pathways are activated, NF-KB inflammatory pathways are suppressed and Nrf2 mediated antioxidant pathways are activated [22, 23, 27]. The clinical evidence is emerging and shows that it is effective in reducing systemic inflammation and early glycemic dysregulation, with a high level of support [4, 5, 35]. Furthermore, with the advent of biogenic nanotechnology and smart nano-delivery technologies, viable strategies for native extracts whose intrinsic pharmacokinetic properties can be enhanced through formulation of highly bioavailable optimized preparations are available [46, 47]. The future of M. oleifera in modern integrative and molecular medicine is sure to be supported by the current gaps in phytochemical standardization and wide clinical trials and validation.

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