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Systematic Review

A 5-Year Systematic Review (1 November 2019 to 31 October 2023) on the Benefits of the "Miracle Tree" (*Moringa oleifera*)

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Abstract

Moringa oleifera (MO), known for its rich nutritional content and diverse health benefits, has gathered increasing scientific interest over the last 5 years. Thus, this systematic review aims to comprehensively examine recent updates on the beneficial components, health effects, and applications of MO. A systematic search on the benefits of MO was conducted using PubMed as the source database, from 2019 to 2023. Out of 120 articles identified, 27 articles were included in this review after filtration. A total of 3 themes were deduced after analysing the articles; namely, (a) nutritional, antioxidant, and phytochemical content, (b) health benefits and disease prevention, and (c) food fortification and quality analysis. Thematic analysis highlights the variability in the nutritional, antioxidant, and phytochemical content of MO, which can be influenced by several factors. MO also demonstrated beneficial effects in various health conditions, although these effects may depend on the dosage of MO. Moreover, MO may be a promising resource for food fortification.

Keywords: *Moringa oleifera;* Miracle Tree

Introduction

Natural botanical plants have been used since the beginning of human civilisation to prevent and treat diseases. *Moringa oleifera* Lam. (MO), otherwise known as the "Tree of Life" or "Miracle Tree", is a species from the Moringaceae family that originates from Afghanistan, India, Bangladesh, and Pakistan [1]. Other common names include Drumstick tree, Benzolive tree [2] and Kelor [3]. It is a fast-growing and drought-resistant tree that thrives in almost all tropical and subtropical regions, thus making it widespread across the globe [4]. Historical usage of MO dates back to 150 B.C. when ancient warriors who defeated Alexander the Great drank MO leaf extract as an elixir to increase power and ease pain [4].

Every part of the MO plant; such as the leaves, barks, pods, seeds, flowers, stems, and roots; has a variety of uses [4]. For instance, MO seeds have been used for water purification, cooking oil, and flour, while the leaves are widely consumed to improve nutritional status. MO is recognized as a natural source of vitamins B1, B2, B3, C, E, calcium, magnesium, phosphorus, potassium, iron, antioxidants, essential amino acids, carotenoids, flavonoids, and phenols [5]. These compounds are essential for fighting malnutrition, especially in developing countries where malnourishment is a major concern for nursing mothers and infants [6, 7]. Other potential benefits; such as anti-inflammatory, antioxidant, anti-tumour,

antidiabetic, anti-hypertensive, cholesterol-lowering, antibacterial, antiviral, antispasmodic, anti-asthma, hepatoprotection, and immunomodulatory effects; were also noted [1, 3-5]. Due to its extensive nutritional and health properties, several studies have explored its effects when fortified into food products and used as a nutritional supplement. A review by Islam Zahidul., *et al*. reported the use of MO leaves as medicinal capsules or tea [5]. Similarly, Trigo., *et al*. reviewed the successful fortification of MO into food products [7]. For instance, MO has been incorporated into bakery products, snacks, beverages, dairy products, and meat products to enhance its nutritional, antioxidant, and antimicrobial capacity. However, negative changes in sensory attributes such as flavour, colour, texture, and volume were reported in most products unless MO was incorporated in very small doses.

The number of studies on MO has doubled over the past 5 years with 5 systematic reviews to date (Table 1). However, these systematic reviews analysed the efficacy of MO in mitigating specific health conditions, with only one systematic review comprehensively evaluates the chemical components and health benefits of MO [8]. In this systematic review, we aim to take a broader approach by consolidating findings from the last 5 years on the beneficial components, health effects, and application.

Aims	Main Findings
Evaluate the phytology, phytochemistry, traditional usages, pharmacology, toxicity, agricultural economy, nutritional effects and future outlook of MO [8]	Identified chemical constituents from different parts of MO. Pharmacological activities of MO include anti-tumour, antidiabetic, anti-hypertensive, cholesterol-lowering, antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antispasmodic, anti-asthma, antiurolithiatic, anthelminthic, hepatoprotection, immunomodulatory effects. Other applications include abortion, contraception, reducing ulcers, inhibition of platelet aggregation, and enhanced blood clotting. MO is generally toxic at very high doses, like 600mg/kg. Further *in vivo* studies and clinical trials are required to confirm the effects and efficacy of MO.
Assess the effectiveness of oral galactagogues, including MO, for increasing production of milk in non-hospitalised breastfeeding [9]	MO, as a natural oral galactagogue, has shown promising results, with some indicating a potential increase in infant weight and milk volume. However, studies that indicated no beneficial results were also present. It remains inconclusive and further research is required.
Assess the effects and efficacy of therapeutic plants, including MO, in cerebral ischemia-reperfusion injury [10]	MO, along with other traditional herbal medications, has emerged as a promising adjuvant therapy for improving neuroplasticity after cerebral ischemic/reperfusion injury. However, further research is required to elucidate the exact mechanisms of MO and dosage requirements for treatment.
Assess the recent studies of MO and its possible antioxidant and anti-inflammatory mechanisms of action in cardiometabolic disorders [11]	MO demonstrated antioxidative and anti-inflammatory effects in animal studies with cardiometabolic disorders through various mechanisms. Relatively high safety profile, and potential dose-effect relationships in the reduction of glucose, blood pressure and BMI levels. Further research on clinical studies is required to confirm efficacy and safety.
Assess the effectiveness and beneficial results of MO extracts or phytochemical derivatives against middle cerebral artery occlusion *in vivo* models of ischemic stroke [12]	Ethanolic extract of MO, along with α-pinene showed promising results in reducing ischemic infarct volume caused by middle cerebral artery occlusion in animal models. showed an ability to reduce infarct volume and malondialdehyde (MDA) levels while increasing antioxidant enzyme levels like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). Further clinical trials are required to confirm their efficacy in the cerebroprotective effects.

Table 1: Previous systematic reviews on MO.

Methods

A PubMed systematic literature search was performed on 16 January 2024, to identify articles on the benefits of MO published from 1 November 2019 to 31 October 2023. The search was conducted using the search term "moringa AND benefi*". The following exclusion criteria were applied: (a) non-English articles were excluded; (b) articles that were retracted were excluded; (c) articles with no access to full-text were excluded; (d) secondary research articles, including narrative reviews, systematic reviews, and meta-analysis, were excluded; (e) articles that were not related to human health were excluded; (f) articles that were not related to nutrition were excluded; (g) articles that did not include MO as the main study were excluded.

Results

A total of 120 articles were retrieved from PubMed (Figure 1); of which, 27 articles were included in this review, with 3 themes identified (Table 2).

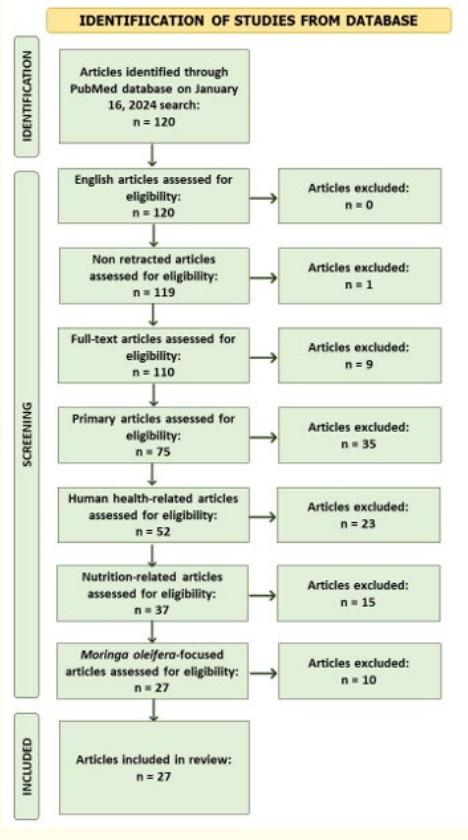

Figure 1: Process of study selection using PRISMA.

Theme	References	Number of Articles	Percentage of Themes
Nutritional, antioxidant, and phyto-chemical content	Nutritional: [13–16], An-tioxidant and phytochemicals: [13–15,17–20]	8	29.63%
Health benefits and disease prevention	Neurological: [14,19], Immune-related: [16, 21–23], Liver and kidney: [24–26], Cancers: [27–29], Haematology: [26,30,31], Reproductive: [32,33], Gastrointestinal: [26,34], Other: [30,35–37]	20	74.07%
Food fortification and quality analysis	[17,30,38,39]	4	14.81%

Table 2: Thematic classification of articles on the health benefits of MO.

Theme 1.1: Nutritional content

Several studies reported that the protein content accounts for at least 25% of MO leaf powder and 20% of crushed MO leaf (Supplementary Table S1). A higher content was observed in seeds, with protein accounting for at least 35% of crushed MO seeds (Supplementary Table S1). A total of 18 amino acids out of the 20 available amino acids were identified by Fejér., *et al.* [15]. The highest content of amino acids identified include glutamic acid (33.8g/kg), aspartic acid (23g/kg), leucine (22.9g/kg), and arginine (21.4g/kg). The reported protein and amino acids content, such as lysine, leucine, isoleucine, valine, threonine, and tryptophan, met at least half of the recommended daily intake recommended by the United States Food and Drug Administration and World Health Organization [15].

The total fat content of MO leaf powder varies between studies (Supplementary Table S1), with reports of a low [13,16] to medium

[1]https://pubmed.ncbi.nlm.nih.gov/?term=moringa+AND+benefi*&filter=dates.2019/11/1-2023/10/31

fat content [14]. Gambo., *et al*. stated that MO leaf powder contains a large proportion of monounsaturated fat [16]. Contrastingly, Peñalver., *et al*. reported that MO leaf powder contains a high proportion of polyunsaturated fat, like omega-3 and omega-6 [13]. This suggests that the fat content mainly consists of unsaturated fat. A total of 13 fatty acids were identified in the leaves and seed extracts by Fejér., *et al*. [15]. A difference can be observed in the content of oleic acid, the highest content of fatty acids identified in the extract of MO seeds (76.78 ± 0.20%) and leaves (25.01 ± 0.13%). However, MO leaves have a more balanced ratio of oleic, palmitic (24.84 ± 0.10 %), and linolenic (24.71 ± 0.21%) acid, while MO seeds are predominantly made up of oleic acid with very low quantities of other fatty acid.

The carbohydrate and dietary fibre content among the filtered articles varies (Supplementary Table S1), with Peñalver., *et al*. and González-Burgos., *et al*. describing that the values attained from their studies were different from other studies [13,14]. Peñalver., *et al*. stated that fibre content may have been included in the total amount of carbohydrates, causing the variation in value [13]. For mineral content, there were also variations between the filtered articles (Supplementary Table S1). Among them, potassium (K), calcium (Ca), sulphur (S), and iron (Fe) were found to be the most abundant (Supplementary Table S1). Other minerals like magnesium (Mg), phosphorus (P), boron (B), zinc (Zn), copper (Cu), and manganese (Mn) were also present in MO leaf powder. Additionally, Peñalver., *et al*. reported high levels of folate and notable amounts of carotenoids, vitamins B_1, B_2, A, and C. The bioavailability of minerals was evaluated by Peñalver., *et al*. [13]. In the oral phase, the mineral with the highest bioavailability was sodium (Na) while the lowest was Zn. The mineral with the highest bioavailability in the gastric phase was P and the lowest was Fe. Lastly, Ca is the mineral with the highest bioavailability while Fe was also the lowest. Therefore, despite MO leaf powder being rich in Fe, it might not be efficiently absorbed into the body. Peñalver., *et al*. stated that as Fe in MO is mainly made up of non-heme forms, it may be chemically complex with various inhibiting factors like phytate, polyphenols, or dietary fibres [13]. Contrastingly, it was found that Ca bioavailability increases in the intestinal phase when compared to the gastric phase. Peñalver., *et al*. explain that the formation of soluble and stable complexes when Ca interacts with digestive secretions or proteins could improve the bioaccessibility of Ca [13].

The nutritional content may change due to a variety of factors, including the analytical method used, maturity, weather, time, and method of harvest [13]. This could also lead to the variation seen in the total fat, carbohydrates, dietary fibre, and mineral content among the filtered articles.

Theme 1.2: Antioxidant, and phytochemical content

The antioxidant capacity of MO extracts (Supplementary Table S2) was analysed by various methods, namely the test of scavenging activity against 2,20-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid radical (ABTS+), ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), superoxide radical, and hydroxyl radicals [13-15, 17, 18]. Variations in the antioxidant activity were seen among different methods, with Peñalver., *et al*. reporting modest antioxidant activity as compared to extracts from other Indian plants [13]. Generally, strong correlations were observed between the antioxidant activities and the phenolic compounds present [13, 15, 18]. Llorent-Martínez., *et al*. also reported individual compound activity can also influence overall activity [18]. For instance, the study reported that S3 (capsule supplement containing 500mg MO leaves along with chromium, picolinate, magnesium, and stearate) has lesser overall phenolic compounds but has a high quercetin-O-hexoside content as compared to S6 (tablet supplement containing 490mg MO leaves). Yet, both displayed similar antioxidant activity.

108 phytochemicals were identified to be present in MO extracts (Supplementary Table S3), mainly consisting of flavonoids, phenolic acids, and other compounds [15,17-20]. Specifically, kaempferol and quercetin derivatives are highlighted as the main flavonoid compounds [18,19]. The total phenolic content (TPC) varies among the different extracts (Supplementary Table S4). Several factors influencing TPC include MO harvest time [14,15,18], MO origin [17-19], extraction solvent used [14,15,17], and methods used during processing or extraction [15,17-19].

Theme 2.1: Health benefits and disease prevention: Neurological disorders

Treatment with MO extracts has shown neuroprotective potential against hydrogen peroxide-induced oxidative stress. Gao., *et al*. analysed the neuroprotective effects of polyphenols from MO, specifically isoquercitrin and astragalin, against oxidative stress in rat neuroblastoma (PC-12) cells [19]. Contrastingly, González-

Burgos., *et al*. investigated the neuroprotective potential against oxidative stress on human neuroblastoma (SH-SY5Y) cells [14].

Exposure to hydrogen peroxide significantly reduced cell viability in both studies, with Gao., *et al*. reporting exposure to increasing concentrations of hydrogen peroxide from 400 to 1200nM for 12h decreased cell viability in a dose-dependent manner [19]. However, treatment with MO extract, astragalin, and isoquercitrin significantly increased the viability of PC-12 cells in a dose-dependent manner. At the concentration of 6.25µg/mL, MO extract increased cell activity from 44% to 59%. As the concentration increase, cell activity significantly increases ($p < 0.01$), to 78% at 25µg/mL. A similar reaction was seen for astragalin and isoquercitrin. Additionally, González-Burgos., *et al*. investigation displayed a significant increase in cell viability by 25-30% when there was pretreatment with MO extract at non-toxic concentrations of 5, 10, and 25µg/mL, as compared to hydrogen peroxide-treated cells [14].

González-Burgos., *et al*. also reported that MO extracts were able to prohibit morphological alterations caused by hydrogen peroxide in the SH-SY5Y cells [14]. Similarly, pretreatments with MO extract at non-toxic concentrations were reported to significantly reduce oxidative stress, as evident in the reduction of reactive oxygen species (ROS) overproduction. Furthermore, oxidative stress biomarkers, like lipid peroxidation (expressed as reduced thiobarbituric acid reactive substances (TBARS) levels) and reduced glutathione (GSH) content, worsened after hydrogen peroxide treatment. However, pretreatment with 25µg/mL of MO extract reduced TBARS levels by 69%. Moreover, 25µg/mL of MO extract significantly increased the index redox, the ratio of GSH to oxidized glutathione (IR of 0.168). Hydrogen peroxide also reduced the activities of antioxidant enzymes like CAT, SOD, and glutathione peroxidase (GPx). However, pretreatments with MO extracts at 10 and 25µg/mL restored the antioxidant activities. Hydrogen peroxide-induced oxidative stress also reduced mitochondrial membrane potential (MMP), and increased calcium levels in the cytosolic portion of the mitochondria, causing impairments to the cells. However, pretreatment with 10 and 25µg/mL of MO extract reversed the change in MMP and mitochondrial calcium levels induced by hydrogen peroxide. Similarly, pretreatment with 25µg/mL restored calcium cytosolic levels to the control levels (cells with no extract added).

Theme 2.2: Health benefits and disease prevention: Immune-related disorders

The usage of MO has shown positive effects on immune-related diseases. Several studies found significant changes in the parameters involved in immunomodulation and improved human immunodeficiency virus (HIV) pathogenesis. Treatments with MO led to significant increases in the clusters of differentiation 4 (CD4) T-lymphocyte cell counts in HIV-positive (HIV+) individuals [16,21,22]. Gambo., *et al*.'s study found that CD4 counts in the HIV+ individuals undergoing antiretroviral therapy (ART) who ingested 5g MO leaf powder sachets thrice daily over 6 months were 10.33 times higher than control that ingested the placebo sachets (cornstarch powder with chlorophyll) [16]. Similar findings were reported in the study by Aprioku., *et al*. where HIV+ adults receiving highly active antiretroviral therapy (HAART) were given MO supplement capsules [21]. Interestingly, Minutolo., *et al*.'s study identified that the microRNAs (miRNAs) from MO seed extracts markedly improved the viability and percentage of apoptosis in peripheral blood mononuclear cells (PBMC) in naïve HIV+ individuals, but did not affect healthy individuals [22]. The ability to induce apoptosis was evident in the CD4 expression of apoptosis-related genes, with increased Fas cell surface death receptor (Fas) expression and decreased expression of B-cell lymphoma 2 (Bcl2) genes.

Likewise, Aprioku., *et al*.'s study revealed improvement against haematological abnormalities usually seen in HIV+ patients. The white blood cell (WBC), lymphocyte, neutrophil, haemoglobin, red blood cell (RBC), and platelet counts significantly increased ($p < 0.05$) after 3 months of MO supplementation with HAART, as compared to HAART treatment alone [21]. Minutolo., *et al*. investigation also reported a reduction in inflammation as indicated by the decreased expression of pro-inflammatory cytokines like tumour necrosis factor-alpha (TNF-α). This study also highlighted one specific miRNA, p-miR858, present in MO that inhibits the vav guanine nucleotide exchange factor 1 (VAV1) protein, that is involved in HIV pathogenesis. This shows the potential role of MO supplementation in regulating infectious mechanisms at the post-transcriptional level.

MO treatments also improved immune defects caused by malaria. Pilotos., *et al*. studied the short-term (7 days), long-term (3 weeks),

prophylactic (pre-infection), and curative (post-infection) effects of MO pallets (MO leaf powder) on mice [23]. It was concluded that both prophylactic and curative MO treatments showed a reduction in parasite load, along with increased activation of $CD4^+$ cells. The secretion of pro-inflammatory cytokine that is released as part of the body's immune response to regulate inflammation, specifically tumour necrosis factor (TNF) and interferon-gamma (IFNγ), in $CD4^+$ cells was also increased. However, the increase or decrease in $CD4^+$ activation or cytokine production depends on the dosage and duration of treatment. Long-term treatment at lower doses, which better represents the diet in MO-consuming regions, also showed an increase in the number of activated $CD4^+$ cells and TNF secretion after malaria infection, though it is not significant. Although short-term treatment in pre-infected mice at high doses increased the T-box expressed in T cells (T-bet) expression that is associated with the secretion of proinflammatory cytokines, low doses of MO had adverse effects. A reduction in the activation of effector $CD4^+$ cell counts was seen at short-term low dosages after infection. Notably, MO was found to prevent the immune suppressive effect caused by malnutrition after malaria infection as it was observed to counter the reduction in $CD4^+$ activation.

Theme 2.3: Health benefits and disease prevention: Liver and kidney disorders

MO has been seen to reverse the adverse effects caused by melamine (ML). Abd-Elhakim., *et al*. analysed the prophylactic and therapeutic effects of administrating MO ethanolic leaf extract as compared to ML on rats [24]. ML induced substantial improvements in the activities of hepatic enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphate (ALP). Similarly, an increase in bilirubin levels, urea, and creatine, along with a decrease in protein levels was observed (Supplementary Table S5). However, prophylactic and therapeutic treatments with MO extract significantly reversed these ML-induced effects. Furthermore, improvement was observed in the hepatocellular damage and renal tubular degeneration in ML-treated rats after MO treatments. Additionally, MO treatments were able to improve the antioxidant enzyme activity of CAT and GPx, along with the decrease in apoptosis-triggering genes in hepatic tissues. This effect can also be seen in renal tissues. ML-induced increase in gene expressions; kidney injury molecule-1 (KIM-1) marker, which is important in the removal of cellular debris; tissue inhibitor of metalloproteinases-1 (TIMP-1), which is associated with renal interstitial fibrosis; and TNF-α which is a pro-inflammatory cytokine involved in renal diseases, was improved after MO treatment.

MO was also reported to mitigate the negative effects induced by heat stress (HS) treatment. Yasoob., *et al*. investigated the effects of MO treatment on heat-stressed liver tissue response in rabbits [25]. Similar to the previous study, HS treatment led to elevated levels of hepatic enzyme activities, specifically ALT, AST, and gamma-glutamyl transferase (GGT) (Supplementary Table S5). The metabolic parameters like cholesterol, creatinine, blood urea nitrogen (BUN), and glycated serum protein (GSP) were also markedly increased. Lastly, HS altered the oxidative stress marker of MDA, diamine oxidase (DAO), SOD, glutathione S-transferase (GST), and CAT. Nonetheless, MO treatment displayed improvements in all adverse effects induced by HS treatment, with certain parameters even exhibiting better results than control.

A significant upregulation of genes involved in antigen processing, antigen presentation, and primary immunodeficiency pathways was seen in MO-treated rabbits when compared with control rabbits. But when compared to HS-treated rabbits, MO-treated rabbits exhibited upregulation of genes associated with cellular response to endogenous stimulus, transmembrane receptors, epithelial cell proliferation, along with genes involved in the pathway for mineral absorption and unsaturated fat synthesis. MO-treated rabbits also had significantly upregulated expressions of antioxidant transcription factors, such as nuclear factor erythroid 2-related factor 2 (Nrf2), along with genes associated with immune responses, like interleukin-6 (IL-6).

The study by Afolabi., *et al*. assessed on the hepatic injury induced by intestinal ischemia/reperfusion injury (IIRI) [26]. MO methanolic leaf extract treatment was found to significantly increase hepatic weight, lower bacterial count, and preserve the histoarchitecture of the liver in rats. Likewise, MO caused a decrease in oxidative stress, inflammation, and apoptosis caused by IIRI. This is evident in the decrease in the levels of oxidative stress marker, MDA; inflammatory markers, such as myeloperoxidase activities (MPO), TNF-α, and IL-6; and apoptotic marker, caspase 3.

Theme 2.4: Health benefits and disease prevention: Cancer

MO leaf powder was noted to demonstrate anticancer potential against various cell lines, including human prostate cancer (PC-3)

cells [27], human osteosarcoma (SaOS-2) cells [28], and human colon adenocarcinoma (HT-29) cells [29]. A decrease in cell viability in a dose-dependent manner was seen in the 2020 studies by Khan., *et al*. and Caicedo-Lopez., *et al*. [27,29]. Yet, the study published in 2022 by Khan., *et al*. stated that at 25 and 50mg/mL of MO, an improved cell viability by 119.5% and 151.82%, respectively [28]. A decrease in cell growth was only observed at the concentrations of 100 and 200mg/mL, to 96.34 and 71.86%, respectively, indicating antiproliferative effects of MO on cancer cells.

Pro-apoptotic effects induced by MO were also reported in the studies. This is evident in PC-3 cells through several mechanisms, such as the increased 40,6-diamidino-2-phenyliondole (DAPI) staining used to determine apoptosis activity; the reduction of MMP; enhancement of intracellular ROS generation; modulation of apoptotic genes; MO-induced cell cycle arrest; and downregulation of genes related to Hedgehog signalling, in a dose-dependent manner [27]. Similarly, increased DAPI staining, enhanced intracellular ROS generation and MO-induced cell cycle arrest were the main mechanisms causing SaOS-2 cell apoptosis [28]. However, it is worth noting that the pro-apoptotic effects were only seen at higher concentrations of 100 and 200µg/ml, while low concentrations of 25 and 50µg/ml instead stimulated proliferation, mineralisation, and expression of genes involved in promoting differentiation of osteoblast. In HT-29 cells, it was noted that MO caused cell death through mechanisms like increased intracellular hydrogen peroxide production, apoptosis, autophagy, and necrosis. These effects could be associated with the colonic antioxidant capacity, high amounts of phenolics and short-chain fatty acids in the fermented non-digestible portion of MO leaf powder at 12 hours of *in vitro* colonic fermentation.

Theme 2.5: Health benefits and disease prevention: Haematological disorders

Several studies suggest MO have positive influences on some haematological parameters. Khanam., *et al*.'s intervention involving fresh MO leaves cooked as pakora resulted in significant positive alterations in the haemoglobin levels among adolescent girls ($p = 0.010$). Nurhayati., *et al*.'s investigation on the effects of MO leaf powder for 12 weeks on blood profile in rats revealed variations in blood profile at different dosages. The dosage of 400mg/kgBW/day displayed better results as compared to 200 and 800mg/kgBW/day. There was an observed increase in the levels of haemoglobin, erythrocytes, hematocrit, leukocytes, and mean corpuscular volume (volume of erythrocytes) at 400mg/kgBW/day, although these increases were statistically insignificant. It is worth noting that the mean corpuscular haemoglobin (MCH) value (average haemoglobin content in erythrocyte) decreased as the dosage increased, though it remained within the normal levels. Lastly, Afolabi., *et al*.'s study reported improvements in the altered blood profile of rats with IIRI, specifically in the WBC and platelet counts after MO treatments [26]. However, the erythrocyte, haemoglobin, and MCH concentrations only improved at a low dosage of 200mg/kg as compared to 400mg/kg.

Theme 2.6: Health benefits and disease prevention: Reproductive disorders

MO offers several health benefits to the reproductive system, specifically in erectile dysfunction (ED) [32] and polycystic ovary syndrome (PCOS) [33]. Oyeleye., *et al*. examined the effects of MO seeds- and leaf-supplemented diets on enzymes related to erectile dysfunction (ED) of streptozotocin-induced diabetic rats [32]. It was found that the TBARS level, a lipid peroxidation marker, was significantly decreased ($p < 0.001$) in both groups of diabetic rats that were fed with MO seeds and leaves, respectively, with or without ED drug treatment by acarbose (ACA). Notably, treatments with ACA with MO leaves for 14 days were significantly better ($p < 0.05$) as compared to treatment with ACA alone. The thiol levels that measure redox status in MO-treated diabetic rats were also upregulated, with or without ACA, with the groups that were fed with MO treatment at 4% (seeds or leaf) with ACA being the highest. In addition, the enzyme activities associated with ED decrease after MO treatment, with or without ACA. For instance, diabetic rats treated with MO seeds or leaf treatments with ACA had decreased arginase, phosphodiesterase type 5 (PDE-5), acetylcholine (AchE), monoamine oxidase (MAO), angiotensin-converting enzyme (ACE), and adenosine deaminase (ADA) activities. Hence, ACA with MO may be beneficial in diabetic-induced ED. Another study by Wu., *et al*. explored the impact of MO leaf powder on PCOS in letrozole-induced rats [33]. An improvement in the oestrous cycle and ovarian morphology was observed, as evident in the decrease in the atretic follicle, number and size of the vesicle, along with the increase in granulosa cell size and thickness. Sex hormones were also better regulated under MO treatment, with decreased serum testosterone

and luteinizing hormone levels alongside increased estradiol and follicle-stimulating hormone (FSH) levels. MO treatment exhibited protective effects against diseases related to the pathogenesis of PCOS. This includes improved insulin resistance, gut barrier function, intestinal microbiota, decreased oxidative stress, and inflammation. Thus, the findings from this study suggest that MO treatment could potentially be favourable for the management of PCOS.

Theme 2.7: Health benefits and disease prevention: Gastrointestinal disorders

The study by Afolabi., *et al*. assessed the effects of MO on intestinal injury in rats induced by IIRI [26]. After MO methanolic leaf extract treatment, there was a substantial increase in intestinal weight, a reduction in bacterial count, and preservation of the histoarchitecture of intestinal cells. Additionally, improvements in oxidative stress marker, inflammatory markers, and apoptotic marker were observed. The polysaccharides in MO also showed positive influences on intestinal morphology, serum indexes, caecal microbiota, and the metabolic profile of the small intestine in mice [34]. Notably, the jejunum, villi length and crypt depth were significantly improved ($p < 0.05$) after MO treatment. MO also resulted in significantly decreased ($p < 0.05$) levels of glucose. In higher concentrations at 60mg/kg, total cholesterol and MDA significantly decreased ($p < 0.05$), while SOD and CAT markedly increased ($p < 0.05$). Moreover, positive alterations in the caecum microbiota were seen in MO-treated rats. The analysis of the metabolic profile of small intestinal contents showed MO treatment affecting 14 pathways. Among them, 4 pathways were significantly enriched, including the metabolism of amino sugar with nucleotide sugar, fructose with mannose, pantothenate with coenzyme A (CoA) biosynthesis, and pyrimidine metabolism.

Theme 2.8: Health benefits and disease prevention: Other disorders

Besides the above-mentioned disease prevention effects, several effects against other diseases such as the prevention of malnutrition, diabetes mellitus, improving insomnia, and myocardial injury have also been reported.

Khanam., *et al.*'s investigation on the effects of MO-fortified snacks on underweight status in female adolescents in rural Bangladesh noted that a higher increase in height and weight was observed, although not significant [30]. Likewise, serum retinol levels were significantly improved ($p = 0.00$) after consumption of MO leaf along with normal staple food. This shows the potential of MO to ensure the proper development of children and the prevention of malnutrition in rural areas.

In Zainab*., et al.*'s study, the top 5 phytochemical constituents present in MO extracted from the PubChem database were evaluated and compared against mutated protein insulin receptor kinase associated with diabetes using *in-silico* molecular docking techniques [35]. The molecular interaction pattern between the mutated protein and each phytochemical was analysed, with results showing effective receptor binding between all phytochemicals and the mutated protein. This suggests that the phytochemicals in MO can potentially treat diabetes mellitus.

Cold-pressed MO seed oil and its bioactive components, such as oleic acid, β-sitosterol, and stigmasterol, demonstrated significant improvements in sleeping behaviours in rats [36]. This was evident in the reduction in the locomotion activity, and sleep latency, along with increased hypnotic activity and sleep duration. Anti-seizure effects were also noted in MO and its bioactive components, displaying a dose-dependent protection against pentylenetetrazol-induced convulsions and a significantly reduced mortality rate. These findings indicated MO's ability to treat insomnia and prevent convulsions.

Finally, Li., *et al*. analysed the protective effects of MO against the myocardial damage caused by myocardial infarction (MI) in mice. A higher survival rate was observed in the mice that had taken the food supplemented with 900mg of MO seed powder for 6 weeks, while no difference in survival rate was seen for the mice that consumed 600mg of MO seed powder. All MO-treated groups observed improved left ventricle (LV) ejection fraction, improved LV fractional shortening values, significantly smaller heart volume, thicker LV anterior wall, smaller volume of infarcted heart, significant reductions in fibrotic scarring, reduced MI-induced myocardial apoptosis, and decrease MI-induced oxidative and nitrosative stress. This suggests the significance of MO in ameliorating myocardial injuries caused by MI.

Theme 3: Food fortification and quality evaluation

MO fortification into daily food and commercial products has been evaluated based on its effectiveness in dietary and health

benefits, along with the consumer's perception of the product. This includes tea [17], snacks for school-going children [30, 38], and gluten-free bread [39].

MO has been shown to have an abundance of health-promoting properties, as evidenced by its TPC, TFC, antioxidant [17, 38], micronutrients [30,38,39], and macronutrients [39]. For instance, the study by Khanam., *et al*. in 2022 observed significant improvements in the haemoglobin ($p < 0.009$) and retinol ($p = 0.000$) levels in female adolescents in rural Bangladesh after consumption of MO leaf-fortified pakora (an oil-fried snack) [30]. Similarly, Adi., *et al*.'s study on the effects of MO leaf-fortified functional crispy rice snack for school-age children in food-insecure regions showed that other than protein and zinc levels, the vitamin A, C, calcium, and polyphenol content increases as MO concentration increases [38]. Interestingly, the hedonic test results showed that the crispy snack recipe with a lower concentration (10%) of MO leaf puree and the higher concentration (20%) were better accepted as compared to control snacks without fortification. Thus, the fortification of functional food in the form of snacks for school-going children and adolescents can potentially prevent malnutrition and promote growth during puberty in rural or food-insecure areas in the world.

Other than functional snacks, Peñalver., *et al*.'s development of MO-fortified gluten-free sourdough created from 3 different pseudocereals (quinoa, amaranth, or brown flour) has increased levels of total fat, protein, and fibre [39]. However, the addition of MO into all gluten-free sourdough saw a reduction in carbohydrate levels. MO-enriched bread was also not as accepted as non-enriched bread samples made from just pseudocereals. Nevertheless, gluten-free bread enriched with MO was still better accepted than commercial gluten-free bread.

The presence of antioxidant, phenolic and flavonoid content was also found in MO tea supplements [17]. Specific phenolic content and composition in MO may influence the consumer's perception of the product. Rodrigues., *et al*.'s study reported that the presence of quercetin-3-O-rutinoside in one of the tea samples contributed to a sweet, floral, and soft flavour, which was most preferred and accepted by consumers [17]. Contrastingly, samples with higher antioxidant values were less favored, as they appeared green, inhomogeneous, and had herbal flavours due to the presence of 3-O-feruloylquinic acid and kaempferol derivatives. Terms associated with MO tea consumption include "health", "wellness", "relaxation", and "leisure", highlighting positive consumer perception of MO tea consumption.

Discussion

The findings from this systematic review show that MO has notable nutritional, antioxidant and phytochemical content, though the results vary within the studies. As expected, when compared with a similar systematic review previously published by Liu., *et al*. the macronutrients and micronutrients value also varies [8]. However, the findings from this review show that different types of treatment to the same part of the plant may cause a difference in the nutritional value. For instance, crushed or powdered leaves (Supplementary Table S1) saw a difference in protein, fat and vitamin C values. Similarly, other factors that may affect the nutritional content that was previously discussed in the results could also cause differences in nutritional value. Additionally, the values in different studies use different reporting units of measurement. The presence of antioxidants and phytochemicals was also mentioned in Liu., *et al*. review [8], with 163 different phytochemicals identified as compared to 103 identified in this review (Supplementary Table S3). This could be due to the limited studies found evaluating other parts of MO over the last five years. For instance, no recent studies were conducted on MO seeds, roots, flowers, pods, stems, and barks, while they were included in the previous systematic review [8]. Moreover, the findings of this systematic review showed beneficial effects in several diseases. In general, most of the findings were new and have yet to be reported in previous systematic reviews. However, dosage seems to matter in some diseases like malaria-induced immunosuppression, osteosarcoma, and haematological disorders. MO is generally acceptable to be used for food fortification for snacks, which can greatly help school-going children or people who are at risk of malnourishment in developing countries, like pregnant women, and the elderly. However, further development may be needed for gluten-free bread and tea to explore the perfect formulation that is most favourable for consumers.

The strength of this review includes the consolidation of all the recent findings of MO's beneficial components, health effects, and applicability in food fortification and supplementations.

This provides an updated and comprehensive view of the recent advancements and discoveries on the topic. However, there are several limitations (Supplementary Table S6). Firstly, the papers included in the findings have their limitations, which could result in biases in results, reduced generalizability, and increased risk of errors. Next, the reliance on the PubMed database as the only source of findings limits the range of research, which can potentially lead to gaps. Hence, multiple databases can be used in future studies to ensure a broader range of research. Likewise, the findings in the nutritional, antioxidant, and phytochemical content of MO may be hard to generalise as most studies used MO from different origins or different reporting units of measurement. This causes incomprehensive conclusions and inconsistency in the findings. Therefore, future studies may look into studies with similar methodologies, reporting measurements, or MO from a single origin to facilitate the comparability and generalizability of findings. Lastly, most studies were conducted in animals or on cell lines. The studies conducted on humans were also limited, therefore further research in clinical studies is required to confirm the findings.

Conclusion

MO is rich in nutritional, antioxidant, and phytochemical content, despite variations in the findings. While continuous research in MO in recent years shows new findings on its effects on a variety of diseases and its potential for food fortification, further research is required to confirm the findings of the health effects and develop better formulations that can meet the preferences of consumers.

Supplementary Materials

Screening of articles by inclusion/exclusion criteria for this study; and supplementary tables S1 (Nutritional content of MO), S2 (Antioxidant capacity analysed), S3 (Phytochemical compounds identified from filtered articles), S4 (TPC analysed), S5 (Percentage changes in serum markers relative to control), and S6 (Limitations and possible solutions for the articles identified); can be downloaded from https://bit.ly/Moringa_oleifera_SR.

Conflict of Interest

The authors declare no conflict of interest.

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