

Immunomodulatory Effect of *Moringa oleifera* and *Mentha spicata* leaves, *ex vivo* and *in vivo* Study

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ABSTRACT

The immunomodulatory properties of *Moringa oleifera* and *Mentha spicata* are of great interest because of their ability to enhance immunity, suppress inflammation, and protect against viruses and other pathogens. This study presented a discussion of the mechanisms involved with *M. oleifera* enhancing immunity through its effects on white blood cell and lymphocyte production as well as modulatory effects on cytokines. Simultaneously, the anti-inflammatory and antimicrobial properties of *M. spicata* were discussed with particular reference to the active ingredients in its essential oils, polyphenols and terpenes that altered immune function. Both herbs had synergistic mechanisms to alter immune function, with *Moringa* enhancing the immune response and *Mentha* modulating the inflammatory response. Pre-clinical studies suggested immune-modulating properties indicative of clinical potential as therapeutic interventions to address infection, autoimmunity and immunosuppression, while larger clinical research studies needed to be conducted to support any of the results. The combination of these plants had promise as an adjunct immunotherapy for the development of safer, natural alternatives to synthetic immunomodulators for use in the context of personalised medicine and integrative health care models. The current research article underscored the necessity for further investigation to enhance the comprehension of the immunomodulating capabilities of these natural products in diverse populations.

Key words: Antioxidant, cytokine, immunomodulators, *Moringa oleifera*, *Mentha spicata*

INTRODUCTION

Immunomodulation is the process of controlling the immune system's responses to meet the body's needs. This keeps the immune system in balance and allows it to fight off pathogens while protecting healthy tissues. (Swaroop *et al.*, 2022; Jan *et al.*, 2024). The immune system can successfully fight off pathogens like bacteria and viruses, thanks to immunomodulation, which also prevents maladaptive reactions like allergies and autoimmunity (Dixit *et al.*, 2017; Mansour *et al.*, 2022; Oriola and Oyediji 2022). A healthy immune system is important for good health because it helps fight infections and keep chronic diseases under control (Mansour *et al.*, 2022). Diseases like HIV/AIDS, age, hunger and psychological stress can all impair immunity and increase a person's risk of becoming ill.

Modern medicine has rekindled interest in plant-based immunomodulators owing to the growing recognition of the limitations and adverse effects associated with synthetic drugs. These natural compounds can effectively modulate immune responses, promote immune homeostasis and provide safer, multifunctional therapeutic alternatives with enhanced biocompatibility (Faheem *et al.*, 2022, Belmouhoub *et al.*, 2024). Many people are looking to natural remedies instead of chemical-based medicines for autoimmune diseases, chronic inflammatory conditions and immunosuppressive diseases because they don't want to deal with the side effects that come with them (Faheem *et al.*, 2022; Swaroop *et al.*, 2022). Recent scientific investigations have substantiated traditional knowledge of medicinal plants by isolating bioactive compounds, such as flavonoids, alkaloids and terpenoids. Modulate both innate and adaptive immune responses,

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enhance immune system homeostasis, regulate inflammatory pathways and offer promising potential as natural, safe and multifunctional immunotherapeutic agents for the prevention and management of various infectious and chronic diseases. Some of these chemicals are flavonoids, alkaloids, terpenoids and other phytochemicals. They work with immune cells to either boost or weaken immune functions, based on what the body needs (Mansour *et al.*, 2022, Gomes and Prado, 2023).

Traditional herbal medicine has involved the use of plants such as *M. oleifera* and *M. spicata*, historically from a range of cultures, for the enhancement of the immune system, healing and reducing signs of inflammation (Misra *et al.*, 2024). These plants contain a wide range of bioactive compounds that can change both innate and adaptive immune responses, which is what makes them useful for immunotherapy, thereby enhancing and strengthening the defense systems of the body against incursion (Das and Saha, 2022; Farinacci *et al.*, 2022; Swaroop *et al.*, 2022; Gomes *et al.*, 2023).

Studies on polyherbal preparations indicate that employing many herbs with synergistic properties might yield more extensive therapeutic outcomes (Gomes *et al.*, 2023). A study on herbal combinations for treating autoimmune diseases indicated that plants with immune-enhancing properties, when paired with anti-inflammatory herbs, yielded superior results in managing inflammation and immune dysregulation compared to their individual use (Swaroop *et al.*, 2022; Sayed, 2023).

Both *M. spicata* and *M. oleifera* have a range of bioactive substances that support their immunomodulatory actions. With antioxidants like quercetin and kaempferol, as well as vital minerals like zinc and iron that promote immune cell proliferation. *M. oleifera*, which is abundant in flavonoids, vitamins, minerals and amino acids, improves immune function. However, *M. spicata* includes flavonoids (such luteolin and apigenin) and essential oils (like carvone and limonene) that increase immune cell activation and control inflammation. When combined, these plants offer a well-rounded and complementary strategy for promoting immunological health by combining inflammation reduction with immune system strengthening.

Both of these plants are known to be good for everyone, there is still a lot of study that needs to be done, especially on how *M. oleifera* and *M. spicata* work together to help the immune system. Many studies have been done on *M. oleifera* to find out, how it can help the immune system, but most of them have been on single chemicals or certain health problems (Mansour *et al.*, 2022). *M. spicata* is also known for its ability to reduce inflammation and kill germs, but its full potential as an immunomodulator has not been fully studied, especially when mixed with other plants (Misra *et al.*, 2024). There haven't been many in-depth studies on how the bioactive chemicals in these plants interact with each other. This study tried to fill that research gap.

The primary objective of this research was to investigate the immunomodulatory mechanisms of *M. oleifera* and *M. spicata*, both individually and in combination, to better understand how they influence immune function. This study aimed at identifying key bioactive compounds responsible for their effects, explore their potential in stimulating and regulating immune responses, and assess their safety and efficacy for use in immune-related therapies. By addressing the existing research gap, this study will provide valuable insights into how these plants can be integrated into modern immunotherapy, offering natural alternatives for immune modulation with potentially fewer side effects compared to conventional treatments (Mansour *et al.*, 2022).

MATERIALS AND METHODS

Fresh leaves of *M. oleifera* (drumstick tree) and *Mentha spicata* (spearmint) were procured from verified sources, guaranteeing purity and quality. The leaves were cleansed, air-dried and meticulously ground into a fine powder. Bioactive substances were extracted using a Soxhlet system with ethanol as the solvent to optimise extraction efficacy. The final extracts were preserved in opaque containers at 4°C for later examination.

To determine the composition of bioactive compounds, phytochemical screening was conducted using preliminary assays involved in the evaluation of alkaloids, flavonoids, saponins, tannins and phenolic substances

employing established biochemical methodologies.

High-Performance Liquid Chromatography (HPLC) was conducted to quantify particular chemicals, including quercetin, kaempferol, carvone and limonene. Thin-Layer Chromatography (TLC) was employed to verify the presence of essential oils and other antioxidants.

The extracts were evaluated for their immunomodulatory activities *in vitro* by the following assays. RAW 264.7 macrophage cells were grown in DMEM with 10% FBS and incubated at 37 °C in a 5% CO₂ atmosphere.

The cells were treated with different doses of *M. oleifera* and *M. spicata* extracts. Cytokine production levels were assessed subsequent to lipopolysaccharide (LPS) stimulation.

An enzyme-linked immunosorbent assay (ELISA) was used to quantify cytokines (e.g., TNF- α , IL-6, IL-1 β), which functioned as indicators of inflammation.

Intracellular ROS levels in treated cells were determined using the DCFDA Cellular ROS Detection Assay. Decreased fluorescence signified the extracts' antioxidant capacity in neutralising reactive oxygen species (ROS).

The *in vivo* immunomodulatory analysis was conducted following ethical guidelines for animal research. Male Wistar rats, weighing approximately 180-200 g, were divided into three groups (control, *M. oleifera*-treated and *M. spicata*-treated), with each group receiving the respective treatment for 14 days. Extracts were administered orally at doses of 200 mg/kg/day selected based on preliminary toxicity assays.

Blood samples were collected on day 0, 7, and 14 to evaluate changes in immune markers. The rats were euthanized for organ collection, specifically the liver and spleen, to assess tissue-level effects.

RESULTS AND DISCUSSION

Alkaloids were found in *Moringa oleifera*, but were absent in *Mentha spicata*. Flavonoids were identified in both the plants, indicating their antioxidant potential. Saponins were absent in both the plants signifying an absence of notable haemolytic and surfactant properties. Tannins were solely detected in *M. oleifera*, perhaps contributing to its astringent and antibacterial properties. Both plants possessed

phenolic compounds, highlighting their capacity to alleviate oxidative stress. Phytochemical analysis revealed that *M. oleifera* contained a broader spectrum of bioactive compounds compared to *M. spicata*, particularly alkaloids and tannins (Table 1).

Table 1. Phytochemical screening of *M. oleifera* and *M. spicata*

Compound	<i>M. oleifera</i>	<i>M. spicata</i>
Alkaloids	Positive	Negative
Flavonoids	Positive	Positive
Saponins	Negative	Negative
Tannins	Positive	Negative
Phenolic compounds	Positive	Positive

M. oleifera exhibited increased levels of flavonoid compounds, specifically quercetin (12.5 mg/g) and kaempferol (9.3 mg/g), thereby reinforcing its traditional application as a medicinal plant with antioxidant properties. Conversely, *M. spicata* possessed higher concentrations of volatile compounds, namely, carvone (14.6 mg/g) and limonene (9.0 mg/g), which were likely accountable for aromatic and potential antimicrobial characteristics (Table 2).

Table 2. Chromatographic analysis of *M. oleifera* and *M. spicata*

Compound	Concentration in <i>M. oleifera</i> (mg/g)	Concentration in <i>M. spicata</i> (mg/g)
Quercetin	12.5	8.2
Kaempferol	9.3	4.7
Carvone	7.8	14.6
Limonene	6.4	9.0

M. oleifera and *M. spicata* demonstrated notable anti-inflammatory benefits by decreasing cytokine levels relative to the control group (Table 3). *M. oleifera* exhibited a more significant reduction in all three cytokines: a 57% reduction compared to a 43% reduction for *M. spicata* in TNF- α , a 40% reduction versus a 30% reduction in IL-6, and a 40% reduction compared to a 20% reduction in IL-1 β , indicating a superior immunomodulatory and anti-inflammatory effect of *M. oleifera* (Table 3).

The experiment found that both *M. oleifera* and *M. spicata* exhibited time-dependent enhancements in ROS scavenging activity. At 0 h, *M. spicata* had a larger initial reaction (20%) than *M. oleifera*, however, after 24 h

Table 3. Inhibition of cytokine production by *M. oleifera* and *M. spicata*

Cytokine	Control (pg/ml)	<i>Moringa oleifera</i> (pg/ml)	<i>Mentha spicata</i> (pg/ml)
TNF- α	35	15	20
IL-6	100	60	70
IL-1 β	50	30	40

(40%) and 48 hours (60%), *M. oleifera* had a higher total ROS scavenging rate. The control group exhibited no ROS scavenging activity, hence validating the effectiveness of the plant extracts. These findings indicated that both the plants exhibited significant antioxidant activities, with *M. oleifera* demonstrating slightly greater efficacy during extended treatment (Table 4).

The experiment demonstrated that TNF- α levels of *M. oleifera* decreased significantly from 35 to 25 pg/ml within 14 days, whereas *M. spicata* levels diminished from 38 to 30 pg/ml in the same timeframe. In cytokine interleukin-6, *M. oleifera* decreased from 100 to 90 pg/ml within 14 days, while *M. spicata* diminished from 110 to 95 pg/mL in the same timeframe. In the cytokine interleukin-10, *M. oleifera* exhibited a minor decrease from 95 to 85 pg/ml, while *M. spicata* demonstrated a progressive reduction from 100 to 90 pg/ml. The findings indicated that both *M. oleifera* and *M. spicata* markedly reduced the elevation of pro-inflammatory cytokines and stabilised IL-

10 levels, implying substantial anti-inflammatory and immunomodulatory effects. Thus, *M. oleifera* showed superior efficacy compared to *M. spicata* in all cytokines, especially in the inhibition of TNF- α and interleukin-6 (Table 5).

The experiment showed that both *M. oleifera* and *M. spicata* reduced inflammation in the liver and spleen tissues compared to the control group. The *M. oleifera* showed a big drop in inflammation to mild inflammation (Grade 1). The *M. spicata* showed improvement, with inflammation dropping to Grade 2, which showed modest anti-inflammatory activity, though not as strong as *M. oleifera*. However, *M. oleifera*, on the other hand, worked better and reduced inflammation in the liver and spleen to a low level (Grade 1) (Table 6).

CONCLUSION

This investigation concluded that *Moringa oleifera* and *Mentha spicata* demonstrated considerable immunomodulatory capabilities, rendering them beneficial for various therapeutic applications. *M. oleifera* was found to boost the development of immune cells, including white blood cells, lymphocytes and natural killer (NK) cells, thanks to its wide range of vitamins, minerals and flavonoids. It changed the levels of cytokines like interleukin-6 (IL-6) and tumour necrosis

Table 4. ROS scavenging activity of *M. oleifera* and *M. spicata*

Time point (h)	<i>Moringa oleifera</i> (%) ROS scavenging	<i>Mentha spicata</i> (%) ROS scavenging	Control (%) ROS scavenging
0	15	20	0
24	40	35	0
48	60	55	0

Table 5. Cytokine quantification (pg/ml) of *M. oleifera* and *Mentha spicata* in blood serum under control and treated condition

Cytokine	Day 0 control	Day 7 control	Day 14 control	Day 0 <i>M. oleifera</i>	Day 7 <i>M. oleifera</i>	Day 14 <i>M. oleifera</i>	Day 0 <i>M. spicata</i>	Day 7 <i>M. spicata</i>	Day 14 <i>M. spicata</i>
TNF- α	40	45	60	35	30	25	38	32	30
IL-6	120	150	180	100	95	90	110	105	95
IL-10	80	100	120	95	90	85	100	95	90

Table 6. Immunological and histological assessments of *M. oleifera* and *M. spicata* in inflammation grade

Organ	Control group (Inflammation grade)	<i>M. oleifera</i> group (Inflammation grade)	<i>M. spicata</i> group (Inflammation grade)
Liver	3 (Moderate inflammation)	1 (Mild inflammation)	2 (Mild inflammation)
Spleen	4 (Severe inflammation)	1 (Mild inflammation)	2 (Mild inflammation)

factor-alpha (TNF- α) and boosted both innate and adaptive immunity. This made it a strong treatment for infections, autoimmune illnesses, and immunosuppression. *M. spicata*, on the other hand, had complimentary mechanisms, including its anti-inflammatory and antibacterial actions, which were caused by its essential oils, polyphenols and terpenes. The plant's ability to lower inflammatory mediators, boosted the activity of immune cells and protected against oxidative stress making it very helpful for treating respiratory infections, chronic inflammation and problems with the immune system.

Even though *M. oleifera* and *M. spicata* showed promise as immunomodulators, more study and clinical validation are needed. Although preclinical investigations and traditional usage provide significant insights, further clinical trials are necessary to validate the efficacy, safety and appropriate dosages of these plants in human populations. Investigations should concentrate on elucidating the long-term effects of these plants, their interactions with conventional pharmaceuticals and their viability as standalone or supplementary therapy for autoimmune illnesses, persistent infections, and other immune-mediated disorders. Standardised extracts and formulations must be created to guarantee uniform outcomes in clinical applications.

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