Print ISSN : 0972-8813
e-ISSN : 2582-2780

[Vol. 24(1) January-April 2026]

Pantnagar Journal of Research

(Formerly International Journal of Basic and Applied Agricultural Research ISSN : 2349-8765)

G.B. Pant University of Agriculture & Technology
Pantnagar, U.S. Nagar; Uttarakhand, Website : gbpuat.res.in/PJR

PANTNAGAR JOURNAL OF RESEARCH

Vol. 24(1) January-April, 2026

CONTENTS

https://doi.org/10.67063/pjr.vol.24.issue.01.17

Phytochemical characterization and immuno-antioxidant potential of *Moringa oleifera* leaf extract in chicken lymphocytes

ANAMITRA RUJ, SONU AMBWANI* and LATA PALIWAL

Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar-263145 (U.S. Nagar, Uttarakhand)
**Corresponding author's email id: sonuambwani@yahoo.co.in*

ABSTRACT: *Moringa oleifera* Lam., renowned for its diverse phytochemical composition and therapeutic properties, is increasingly recognized as a natural antioxidant and immunomodulatory agent. This study investigated the phytochemical profile, antioxidant potential and immunomodulatory action of an aqueous leaf extract of *M. oleifera* (MOE) using chicken splenic lymphocytes *in vitro*. Preliminary qualitative screening confirmed the presence of major bioactive constituents, including tannins, flavonoids and phenols. Quantitative estimation indicated substantial amounts of total phenolics (173.6 mg GAE/g) and flavonoids (127.7 mg RE/g). The extract showed strong free-radical scavenging potential in the DPPH assay, with an IC_{50} value of 56.15 µg/ml. Based on the MTT assay, the maximum non-cytotoxic dose (MNCD) for chicken lymphocytes was determined to be 250 µg/ml. At this dose, MOE significantly promoted both T- and B-lymphocyte proliferation in response to mitogens (PHA, Con A and LPS), indicating pronounced immunostimulatory effects. Additionally, MOE treatment reduced lipid peroxidation and enhanced intracellular antioxidant defences, demonstrated by elevated reduced glutathione (GSH) concentrations along with increased activities of superoxide dismutase (SOD) and catalase (CAT). These findings highlight that aqueous *M. oleifera* leaf extract exerts potent antioxidant and immunomodulatory effects, largely due to its high phenolic and flavonoid content, supporting its potential use as a natural immuno-antioxidant supplement in poultry health management.

Key words: Antioxidant activity, Chicken lymphocytes, Immunomodulation, *Moringa oleifera*, Phytochemicals

Moringa oleifera Lam., a member of the Moringaceae family and commonly known as the "miracle tree," is a rapidly growing, drought-resistant tropical species native to the Himalayan foothills of India. Today, it is extensively cultivated across Asia, Africa and Latin America (Pareek *et al.,* 2023). The leaves, seeds, flowers, bark, roots and pods of the plant are extensively utilized in traditional medicine and human nutrition, reflecting their richness in nutrients and bioactive compounds (Pareek *et al.,* 2023; Bhattacharya *et al*., 2018). The primary phytochemical groups identified in leaf extracts include flavonoids and phenolic acids, with compounds such as quercetin, kaempferol, rutin, catechin, gallic acid, and chlorogenic acid being commonly reported (El-Sherbiny *et al.,* 2024). In addition, both leaf and seed tissues are abundant in vitamins, minerals, carotenoids and fatty acids- notably vitamin C, β–carotene (provitamin A), vitamin E, iron, calcium and essential fatty acids- enhancing their overall nutraceutical significance (Nazim *et al.,* 2025). The ethnomedicinal importance of *M. oleifera* is well recognized in Ayurvedic and other traditional medical systems, where it has long been employed as a remedy for over 300 health conditions (Bhattacharya *et al*., 2018; Saini *et al*., 2016). Extracts from *M. oleifera* leaves have been shown to enhance innate immune responses, thereby strengthening host defence against pathogenic challenges (Kaleo *et al*., 2019).

Plant-derived polysaccharides- such as glucans, mannans, pectins, fucoidans, galactans, fructans and xylans- are key bioactive constituents that exert notable immunostimulatory effects. They act on innate immune cells by inducing cytokine and chemokine release by macrophages and on adaptive immune cells by activating natural killer cells, T lymphocytes and B lymphocytes (Agarwal and Ambwani, 2018a; 2018b; Dobutr *et al*., 2025). Oxidative stress is a major contributing factor in the pathogenesis of several disorders, including neurodegenerative diseases, cancer, cardiovascular ailments, atherosclerosis and inflammatory conditions (Afzal *et al*., 2021). Endogenous antioxidant defences, such as enzymes like SOD and

CAT, along with molecules like GSH, help counteract excessive oxidative stress. Additionally, exogenous antioxidants, such as polyphenols, contribute to maintaining redox homeostasis. The interplay between endogenous and dietary antioxidants sustains a balance between oxidative processes and antioxidant defences, thereby mitigating cellular damage while allowing reactive oxygen species (ROS) to perform essential signalling functions (Darul Raiyaan *et al*., 2024). Numerous *in vivo* and *in vitro* investigations have demonstrated the antioxidant potential of *M. oleifera* leaf extracts, which is largely associated with their abundant phenolic acids and flavonoids (Kumawat and Une, 2024). Based on these findings, the current study examined the immunomodulatory and antioxidant effects of *M. oleifera* aqueous leaf extract (MOE) on lymphocytes derived from chicken spleens.

MATERIALS AND METHODS

Collection of plant material

Authentic plant specimen was procured from Medicinal Plants Research and Development Centre (MRDC), G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Preparation of plant extract

The harvested plant material was thoroughly washed with tap water and then with distilled water, shade-dried, and ground into a fine powder. For extraction, 100 g of *M. oleifera* powder was mixed with 1000 ml of autoclaved distilled water and incubated at 37 °C for 48 h in a shaker incubator to achieve uniform extraction. The extract was then filtered successively through muslin cloth followed by Whatman No. 1 filter paper. The resulting filtrate was concentrated using a rotary evaporator at 45 °C to remove excess solvent and subsequently lyophilized to obtain the aqueous extract. The dried extract was stored at "20 °C until further analysis (Ambwani *et al*., 2025). The extraction yield was calculated based on the dry weight of the final extract.

Phytochemical analyses of M. oleifera Leaf extract

To detect the presence of different phytochemicals in MOE, different qualitative and quantitative biochemical analyses were carried out.

Qualitative analysis

The plant extracts were subjected to phytochemical screening to identify the presence of bioactive constituents, following established methods reported by Mir *et al*. (2013).

Tannins were detected by boiling 0.5 g of powdered plant material in 20 ml of distilled water, followed by filtration and treatment with 0.1% ferric chloride ($FeCl_3$) solution. The development of a brownish-green or blue-black coloration indicated the presence of tannins.

Flavonoids were identified by adding a few drops of 1% ammonia solution to the aqueous extract, with the appearance of a yellow colour confirming their presence.

Terpenoids were tested by mixing 5 ml of the aqueous extract with 2 ml of chloroform, followed by the careful addition of 3 ml of concentrated sulfuric acid to form a distinct layer. The formation of a reddish-brown interface signified the presence of terpenoids.

Phenolic compounds were detected by dissolving 500 mg of the extract in 5 ml of distilled water and adding a few drops of 5% neutral ferric chloride. The appearance of a dark green coloration indicated the presence of phenols.

Phlobatannins were assessed by boiling the aqueous extract of dried leaves with 1% hydrochloric acid, where the formation of a red precipitate confirmed their presence.

Quantitative analysis

Total phenolic content (TPC)

Stock solutions of the plant extract and gallic acid were prepared at a concentration of 1 mg/ml. For estimation, 50 µl of the extract solution (100 µg/ml) was mixed with 250 µl of Folin-Ciocalteu reagent and allowed to react for 10 min. Subsequently, 500 µl of sodium carbonate solution

was added, and the reaction mixture was diluted to a final volume of 5 ml using distilled water. The mixture was then incubated for 30 min, after which absorbance was measured at 765 nm using a spectrophotometer. A reagent blank containing all components except the extract was used as the control. The total phenolic content was determined by comparison with a gallic acid standard curve ($y = 0.009x$; $R^2 = 0.9476$), following the method described by Siddiqui *et al*. (2017).

Total flavonoid content (TFC)

Stock solutions of rutin and the plant extract were prepared in distilled water at a concentration of 1 mg/ml. Standard rutin solutions ranging from 5 to 40 µg/ml were prepared and made up to 5 ml with distilled water. For the assay, 5 ml of the extract solution (100 µg/ml) was mixed with 0.3 ml of 5% sodium nitrite ($NaNO_3$). After 5 min, 0.3 ml of 10% aluminium chloride ($AlCl_3$) was added to initiate colour development. The reaction mixture was then incubated at room temperature for 6 min, followed by the addition of 2 ml of 1 M sodium hydroxide (NaOH). The final volume was adjusted to 10 ml using distilled water, and the solution was mixed thoroughly. Absorbance was measured at 510 nm against a reagent blank. Total flavonoid content was determined from a rutin standard calibration curve ($y = 0.0018x$; $R^2 = 0.9655$) and expressed as milligrams of rutin equivalents per gram of extract (RE, mg/g) (Wang *et al*., 2022).

Determination of antioxidative potential by DPPH assay

The antioxidant activity of MOE was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Ahmad *et al*., 2014). Extract solutions of varying concentrations (10–100/ µg/ml) were prepared and 200/ µl of each was transferred into test tubes. To initiate the reaction, 200 µl of DPPH solution in methanol was added to each tube. The reaction mixtures were transferred in triplicate into 96-well plates and incubated in the dark at 30 °C for 30 minutes. After incubation, absorbance was measured at 517 nm using a microplate reader. Ascorbic acid was used as a positive control, methanol as the blank and DPPH solution without extract served as the negative control. The percentage of radical scavenging activity was calculated based on the decrease in absorbance relative to the negative control.

Evaluation of maximum non-cytotoxic dose (MNCD) of MOE in chicken lymphocytes

Spleens were collected from healthy chickens and lymphocytes were isolated aseptically under laminar airflow, following the method of Osman *et al*. (2024). Cells were separated by density gradient centrifugation as described by Rose and Friedman (1976) and viability was assessed using 0.1% trypan blue with a hemocytometer. The viable lymphocyte population was adjusted to 1 × 10w cells/mL in RPMI-1640 media (Himedia, India) supplemented with antibiotics, antimycotics and 10% foetal bovine serum (Himedia, India). Lymphocytes were then exposed to different concentrations of MOE prepared in RPMI-1640, in triplicate, using 96-well tissue culture plates. The MNCD of MOE was evaluated using the MTT assay as per Mosmann (1983).

Lymphocyte proliferation assay

The immunomodulatory activity of MOE was assessed by lymphocyte proliferation (B- and T-cell blastogenesis) assay using lipopolysaccharide (LPS) (Sigma-Aldrich), phytohaemagglutinin-M (PHA-M) (Himedia, India) and concanavalin A (Con A) (Himedia, India) as mitogens, as described by Ambwani *et al*. (2024; 2026) with slight modifications. Lymphocytes (100 µl/well) were cultured in triplicate in 96-well plates with or without MOE and the respective mitogens, and incubated for 68/ hours at 37/ °C in a 5% CO, atmosphere. After incubation, 50 µl of MTT solution (5 mg/ml) was added to each well, followed by an additional 4-hour incubation. The culture medium was then removed, and 100 µl of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals. Absorbance was recorded at 570/ nm using a microplate reader to determine lymphocyte proliferation.

Evaluation of antioxidant potential

Chicken lymphocytes were exposed to the MNCD

of MOE and incubated overnight in a CO, incubator. After incubation, both treated and control cells were collected and cell lysates were prepared for the evaluation of antioxidant parameters.

Lipid peroxidation

Lipid peroxidation was determined by quantifying malondialdehyde (MDA), a secondary product of fatty acid oxidation that forms a chromogenic complex with thiobarbituric acid (TBA). Briefly, 100 µl of cell lysate was mixed with 1.9/ ml of 0.1/ M sodium phosphate buffer (pH/ 7.4) and incubated at 37 °C for 60 min. The reaction was stopped by adding 5% trichloroacetic acid (TCA), and the mixture was centrifuged at 2300 × g for 15 minutes at room temperature to remove precipitated proteins. The resulting supernatant was treated with 1.0/ ml of 1% TBA and heated in a boiling water bath for 15/ minutes. After cooling, absorbance was recorded at 532/ nm. MDA concentration was calculated using a molar extinction coefficient of 1.56 × 10x M^{-1} cm{ ^{1} and expressed as nmol MDA per ml of cell lysate. A reaction mixture without cell lysate served as the blank (De Leon and Borges, 2020).

Reduced glutathione

Reduced glutathione levels were determined following the method of Ambwani *et al.* (2023). In brief, 0.1 mL of cell lysate was precipitated with 0.9 mL of 5% trichloroacetic acid (TCA) and centrifuged at 2300 × g for 15 minutes at 4 °C. Then, 0.5 mL of the resulting supernatant was mixed with 1.5 mL of 0.01% 5,52 -dithiobis-(2-nitrobenzoic acid) (DTNB). The formation of the yellow chromogen was quantified spectrophotometrically at 412 nm, and GSH concentration was calculated and expressed as µmol per mg of protein.

Superoxide dismutase

Superoxide Dismutase activity was evaluated through MTT reduction method. For the assay, 0.65/ ml of phosphate-buffered saline (PBS), 30/ µl of MTT, 10/ µl of cell lysate, and 75/ µl of pyrogallol were combined. The control was prepared without cell lysate, while the blank lacked pyrogallol. The reaction mixtures were incubated at room temperature for 5 minutes. Following incubation, 750 µl of dimethyl sulfoxide (DMSO) was added to solubilize the formazan crystals, and absorbance was recorded at 570/ nm using a blank containing distilled water. Enzyme activity was calculated based on the extent of inhibition of MTT reduction by the sample relative to the control (Badgujar *et al*.,2015).

Catalase

Catalase function was measured according the UV spectrophotometric approach of Azeem *et al.* (2023) with slight adjustments, by tracking the breakdown of hydrogen peroxide at 240 nm. Briefly, 2 mL of buffer solution and 10/ µL of lysate were added into a quartz cuvette, and the reaction started by introducing 1 mL of H_2O_2. The decline in absorbance at 240 nm was monitored every 30 s for 3 min at ambient temperature using a UV–VIS spectrophotometer, against a water reference. Catalase activity was derived from the initial linear velocity of H_2O_2 degradation using the molar extinction coefficient of H_2O_2 and expressed per mg protein.

Nitric oxide assay

Nitric oxide (NO) production was quantified using the Griess reagent (Sigma-Aldrich) in a microplate-based assay (Ambwani *et al*., 2023; 2024). A standard curve was generated using varying concentrations (10–200 µg/ml) of sodium nitrite ($NaNO_2$) (Himedia), which served as the reference for NO estimation ($y = 0.0031x$; $R^2 = 0.9643$).

Statistical analysis

Data analysis was performed with OriginPro 2026 (OriginLab Corporation, USA), experiments were performed in triplicate, and results are presented as mean ± standard error. Statistical differences between control and treated groups were assessed by one-way analysis of variance (ANOVA), with $p < 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Yield percentage

The percentage yield of the lyophilized leaf extract of *M. oleifera* was found to be 8.16% (Fig. 1).

Qualitative analysis of aqueous extract of *M. oleifera* Leaves

The qualitative analysis of phytochemical constituents in *M. oleifera* leaves is presented in Table 1, where (+) denotes the presence and (-) indicates the absence of the respective compounds.

Quantitative analysis of aqueous extract of *M. oleifera* Leaves

Total phenolic content

The total phenolic level of *M. oleifera* extract was calculated using a gallic acid standard curve ($y = 0.0181x$, $R^2 = 0.9476$) and reported as milligrams gallic acid equivalents (GAE) per gram extract. The phenolic concentration was measured as 173.6 mg GAE/g extract.

Total flavonoid content

The total flavonoid amount was evaluated with respect to a rutin standard curve ($y = 0.0018x$, $R^2 = 0.9655$) and reported as milligrams of rutin equivalents (RE) per gm of extract. The flavonoid level in MOE was determined to be 127.7 mg RE/g extract.

Assessment of antioxidant activity using the DPPH assay

MOE exhibited marked antioxidant potential in the DPPH free radical scavenging assay. A clear dose-dependent response was observed, with scavenging activity increasing as the concentration of the extract increased (Fig. 2). The IC_{50} value of MOE was estimated to be 56.15 µg/ml. In contrast, the IC_{50} of the reference antioxidant, ascorbic acid, was 45.27 µg/ml.

Determination of MNCD of MOE in chicken lymphocytes

Table 1: Qualitative phytochemical analysis in *M. oleifera* aqueous leaf extract

S. No.	Phytochemical Analysis	MOE
1	Phenolics	+
2	Flavonoids	+
3	Tannins	+
4	Terpenoids	-
5	Phlobatanins	-

Chicken lymphocytes were exposed to varying concentrations of MOE to determine the MNCD. Cell viability results (Fig. 3) and microscopic observations indicated that cells remained healthy and did not show cytotoxicity up to 250 µg/ml, while higher concentrations caused increased granularity. Cytotoxicity was observed from 375 µg/ml onward. Therefore, 250 µg/ml was established as the MNCD of MOE and used for subsequent analyses.

Lymphocyte proliferation assay

The effect of MOE on lymphocyte proliferation was evaluated using specific mitogens. T cell proliferation was assessed using PHA and Con A, while B cell proliferation was measured with LPS. MOE treatment significantly enhanced T lymphocyte proliferation, with increases of 7.6% (PHA) and 9.2% (Con A) compared to controls. B lymphocyte proliferation also increased by 7.9% in the presence of LPS. These results indicate that MOE stimulates both T and B cell proliferation (Fig. 4).

Evaluation of antioxidant potential

Membrane lipid peroxidation, along with glutathione reductase, superoxide dismutase, and catalase activities, was assessed to determine the antioxidant potential of chicken lymphocytes following treatment with plant extracts. Ascorbic acid was used as the positive control in all assays. The influence of MOE on lipid peroxidation and antioxidant enzyme activities in chicken lymphocytes is presented in Table 2.

A significant ($P < 0.05$) reduction in lipid peroxidation was identified in the MOE-treated group compared with the control, indicating decreased oxidative damage in lymphocytes. The ascorbic acid–treated group also exhibited a significant decline in lipid peroxidation, confirming its protective antioxidant effect. The levels of GSH were significantly ($P < 0.05$) increased in lymphocytes treated with MOE compared to the control group, suggesting an improvement in intracellular antioxidant status. A similar enhancement in GSH content was observed in the ascorbic acid group. SOD activity was significantly ($P < 0.05$) higher in both MOE and ascorbic acid–

Table 2: Effect of *M. oleifera* aqueous leaf extract on LPO, SOD, GSH and CAT activity in chicken lymphocytes

S. No.	Treatment groups	MDA (nm/ml)	GSH (mM/ml)	SOD (units/mg of protein)	CAT(H_2O_2 utilized mM/min/mg of protein)
1.	Control	5.555 ± 0.4235	0.4461±0.015	3.469± 0.0215	50.7782±0.1581
2.	MOE	2.5641 ± 0.0641*	0.523±0.009*	3.656±0.0392*	54.0075±0.4475
3.	Ascorbic acid	1.7094 ±0.4273*	0.4920±0.009*	3.6576±0.0508*	54.0425±0.1775
	SE(m)	0.046	0.011	0.039	0.373

*Significant at P<0.05

treated groups than in the control, reflecting improved enzymatic defence against superoxide radicals. Likewise, catalase activity showed a significant ($P < 0.05$) increase following MOE and ascorbic acid treatments, indicating enhanced detoxification of hydrogen peroxide. The nitric oxide (NO) assay revealed a significant ($P < 0.05$) decrease in NO production in MOE-treated cells (76.989 ± 0.3876 µM) compared to the untreated control group (88.495 ± 1.4466 µM) indicating its antioxidative potential.

Traditional medicinal knowledge is largely oral, and the compounds responsible for the observed therapeutic effects remain largely unknown. Therefore, scientific validation through proper identification and phytochemical characterization is essential to ensure the safety, efficacy, and acceptance of herbal medicines. Plant secondary metabolites are key components of defence systems that protect against environmental stressors and microbial invasion, while simultaneously serving as important sources of pharmacologically active compounds (Ambwani *et al*., 2019; Pandey and Ambwani, 2022). *M. oleifera* has gained considerable scientific attention due to its extensive traditional use and growing evidence supporting its medicinal potential (Kashyap *et al*., 2022). Evidence suggests that *M. oleifera* leaves contain diverse phytochemicals that underpin their biological activity. Aqueous extracts are rich in saponins, flavonoids, terpenoids, cardiac glycosides, and alkaloids, while ethanolic extracts additionally contain tannins, steroids and anthraquinones (Adekanmi *et al*., 2020). Moreover, *M. oleifera* leaves are abundant in polyphenols, flavonoids- especially kaempferol and quercetin- along with tocopherol, phenolic acids and isothiocyanates and carotenoids, which together contribute to their beneficial health effects (González-Romero *et al*., 2020; Kashyap *et al*., 2022; Mohlala *et al*., 2023). Phenolic compounds inhibit key enzymes involved in inflammatory disorders and reduce platelet aggregation by modulating prostaglandin pathways (Okwu and Iroabuchi, 2009). Accordingly, their presence in *M. oleifera* leaves suggests antioxidant,

A. **B.** **C.**

Fig. 1: *M. oleifera* plant material and extract. A) Plant leaves, B) Dried leaf powder, C) Lyophilized aqueous leaf extract

anticoagulant, immune-enhancing and hormone-regulatory potential. Additionally, polyphenols may counter oxidative stress indirectly by generating low levels of hydrogen peroxide (H, O,), which functions in immune regulation and cellular growth (Cory *et al*., 2018; Sultana, 2020).

M. oleifera leaves are commonly used to manage inflammatory conditions because of their high saponin content, as saponins exhibit significant anti-inflammatory activity. The reported efficacy of *M. oleifera* leaf extract in treating inflammatory disorders, including pneumonia, further supports this effect (Hamdy, 2023). *M. oleifera* extracts have been shown to exhibit potent anti-inflammatory, anti-obesity, antioxidant, anti-diabetic and anti-apoptotic activities. These activities are largely attributed to their rich content of flavonoids, polyphenols, particularly quercetin and kaempferol, phenolic acids, caffeoylquinic acid and isothiocyanates (Alkafafy *et al*., 2021; Mohlala *et al*., 2023). In addition, bioactive constituents present in *M. oleifera* extracts act as strong antioxidants by inhibiting free radical formation and propagation, thereby protecting cells from oxidative damage (Mohlala *et al*., 2023).

The antioxidant potential of *M. oleifera* extracts has been widely assessed through the DPPH radical scavenging assay. Notably, crude extracts demonstrated strong activity, with maximum inhibition of 85.08 ± 0.49% at 0.15 mg/L of *Moringa*

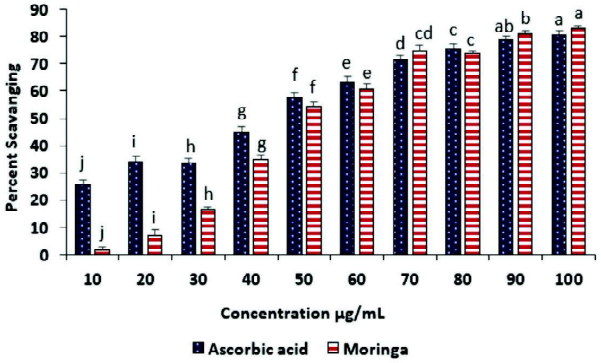

Fig. 2: DPPH radical scavenging activity of *M. oleifera* aqueous leaf extract expressed relative to ascorbic acid as the standard antioxidant. Values with different letters indicate significant differences ($p < 0.05$)

stenopetala leaf extract (Duraisamy *et al*., 2024). Similarly, Saleem *et al*. (2020) reported DPPH inhibition values of 88.50% and 84.46% for methanolic and aqueous extracts of *M. oleifera* at 5 mg/mL, respectively, confirming the strong free radical scavenging capacity of *M. oleifera* leaves. Furthermore, multiple studies have reported a strong association between total phenolic content and the antioxidant activity of *M. oleifera* extracts. The observed protective effects are primarily attributed to the high levels of phenolic compounds, tocopherols and carotenoids present in *M. oleifera* leaves and flower buds (Al-Shebli and Al-Anbari, 2023).

Supporting the immune system through dietary and herbal interventions can beneficially affect gut microbiota, inflammation, viral resistance and nutritional balance (Dong *et al*., 2021). In this context, supplementation of broiler chicks with *M. oleifera* aqueous extract has been shown to improve overall health by enhancing immune function, an effect attributed to its high content of flavonoids and phenolic compounds, including quercetin, kaempferol and rutin (Oyewo *et al*., 2012). The Bursa of Fabricius and spleen are key immunological organs in chickens and immune competence is commonly reflected by lymphocyte proliferation. The Bursa of Fabricius plays a major role in cellular immunity through lymphocyte development, whereas the spleen acts as a peripheral immune tissue abundant in lymphocytes and macrophages (Mohamed *et al*., 2023). Adaptive immunity in birds involves both humoral and cell-mediated responses. Eladia and Ampode (2021) reported that broiler chickens fed *M. oleifera* pod meal exhibited significantly higher spleen and bursa indices than control birds, indicating enhanced immune competence. A higher immune organ index is associated with a stronger immune response and these findings are supported by earlier studies demonstrating that *M. oleifera* supplementation improves nutritional status and immune function in poultry and other animals (Abd El-Hack *et al*., 2018). Measurement of lipid peroxidation (LPO) in tissues is a reliable indicator of oxidative cellular damage. Reduced LPO levels observed in *M. oleifera* extract-

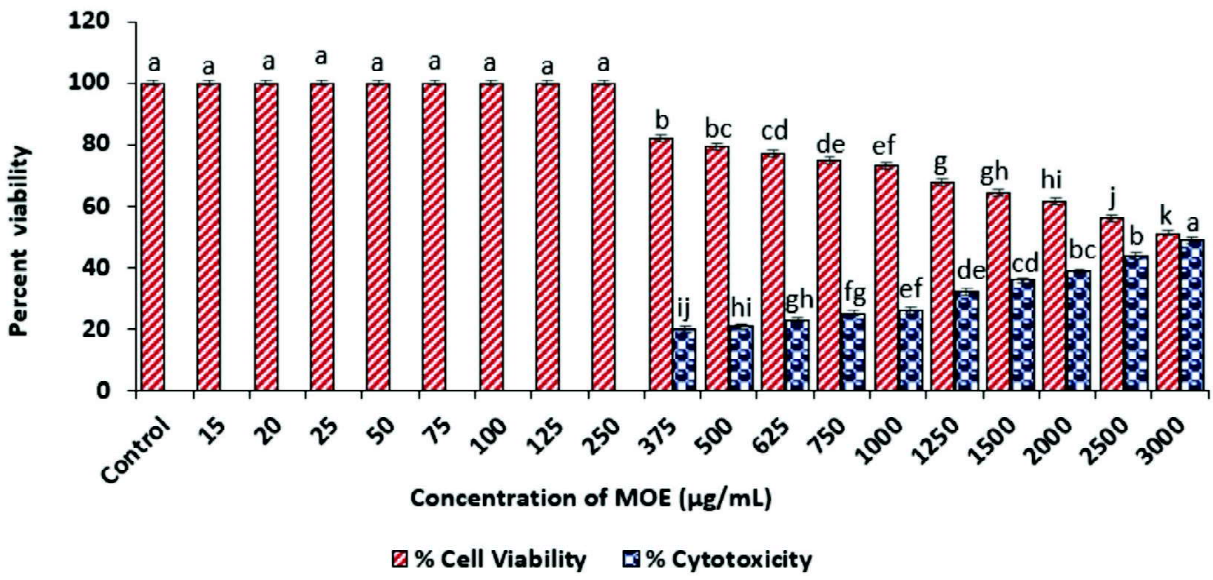

Fig. 3: Percentage viability of chicken lymphocytes following exposure to varying concentrations of *M. oleifera* aqueous leaf extract. Significant differences ($p < 0.05$) are denoted by different superscript letters

treated groups are attributed to its antioxidant and free radical scavenging activities, as well as its ability to restore the biomembranes of hepatic parenchymal cells (Singh *et al*., 2009). Antioxidant defence mechanisms comprise both enzymatic and non-enzymatic components that maintain physiological levels of O, and H, O, . Superoxide dismutase functions as the first line of protection against oxidative damage by facilitating the conversion of superoxide radicals into molecular oxygen and hydrogen peroxide, which is later detoxified through catalase (Salvi *et al*., 2007). Glutathione, an important non-enzymatic tripeptide antioxidant within hepatocytes, performs a vital role in safeguarding membrane protein thiols against injury caused by reactive oxygen species including hydrogen peroxide and superoxide radicals (Singh *et al*., 2014). *M. oleifera* has consistently demonstrated strong antioxidative properties, including increased GSH levels, reduced lipid peroxidation and enhanced antioxidant defence through elevated activities of SOD, catalase, GSH and ascorbic acid across various experimental models (Opuwari *et al*., 2020; Abdel-Aty *et al*., 2025). Additionally, polyphenols and flavonoids present in *M. oleifera* act as direct free radical scavengers, leading to reduced lipid peroxidation and

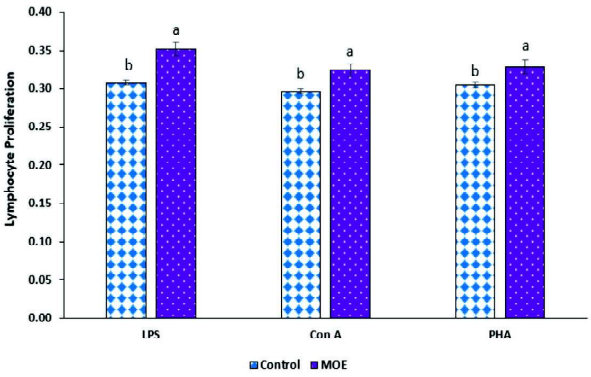

Fig. 4: Effect of *M. oleifera* aqueous leaf extract on mitogen-induced lymphocyte proliferation. Significant differences ($p < 0.05$) are denoted by different superscript letters

protein carbonylation- key markers of oxidative damage- particularly in renal tissues. These protective effects are reflected by decreased malondialdehyde (MDA) levels and increased GSH concentrations, thereby preventing oxidative injury to kidney cells (Akter *et al*., 2021).

CONCLUSION

Overall, the combined data highlight the diverse biological activities of *M. oleifera* as an abundant source containing bioactive molecules with potent anti-inflammatory, immunomodulatory and

antioxidant effects. These actions are primarily linked to its elevated levels of polyphenols, flavonoids, saponins and additional secondary metabolites. The findings not only validate its extensive traditional use but also support its application as a natural feed additive and therapeutic agent in animal health management. Continued research focusing on standardization, dosage optimization and mechanistic insights will further facilitate the effective utilization of *M. oleifera* in evidence-based nutritional and medicinal interventions.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the research amenities provided by the Dean of the College of Basic Sciences and Humanities and the Director of the Experiment Station, G. B. Pant University of Agriculture and Technology, Pantnagar, India. Financial support in the form of Master's thesis research grant awarded to Anamitra Ruj by the Department of Biotechnology, New Delhi, India; which partially supported this work, is also sincerely acknowledged. The authors gratefully acknowledge the Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, for providing the necessary laboratory facilities for the present study.

REFERENCES

Abd El-Hack, M. E., Alagawany, M., Elrys, A. S., Desoky, E. S. M., Tolba, H. M. N., Elnahal, A. S. M., Elnesr, S. S., and Swelum, A. A. (2018). Effect of forage *Moringa oleifera* L. (moringa) on animal health and nutrition and its beneficial applications in soil, plants and water purification. *Agriculture*, 8(9): 145.

Abdel-Aty, D. M., Ibrahim, M. A., Mohamed, S. R., El-Khadragy, M. F., Abdel Moneim, A. E., Fathalla, A. S., and Soliman, D. (2025). Rutin and *Moringa oleifera* leaf extract prevent monosodium glutamate-induced testicular toxicity in adult male albino rats. *Frontiers in Veterinary Science*, 12: 1566471.

Adekanmi, A. A., Adekanmi, S. A., and Adekanmi, O. S. (2020). Qualitative and quantitative phytochemical constituents of moringa leaf. *International Journal of Engineering and Information Systems*, 4(5): 10-17.

Afzal, A., Hussain, T., and Hameed, A. (2021). *Moringa oleifera* supplementation improves antioxidant status and biochemical indices by attenuating early pregnancy stress in Beetal goats. *Frontiers in Nutrition*, 8: 700957.

Agarwal, M., and Ambwani, S. (2018a). Exploring immunomodulatory potential of Niaziminin B found in *Moringa oleifera* Lam. In chicken through *in silico* modeling and Molecular docking studies. *Bulletin of Environment, Pharmacology and Life Sciences*, 7(11): 32-36.

Agarwal, M., and Ambwani, S. (2018b). *In silico* Analysis of Immunomodulatory Potential of Glucomoringinin in Chicken. *International Journal of Pure & Applied Bioscience*, SPI 6(3): 653-659.

Ahmad, S., Ahmad, S., Bibi, A., Ishaq, M. S., Afridi, M. S., Kanwal, F., and Fatima, F. (2014). Phytochemical analysis, antioxidant activity, fatty acids composition, and functional group analysis of *Heliotropium bacciferum*. *The Scientific World Journal*, 2014: 829076.

Akter, T., Rahman, M. A., Moni, A., Apu, M. A. I., Fariha, A., Hannan, M. A., and Uddin, M. J. (2021). Prospects for the protective potential of *Moringa oleifera* against kidney diseases. *Plants*, 10 (12): 2818.

Alkafafy, M. E., Sayed, S. M., El-Shehawi, A. M., El-Shazly, S., Farouk, S., Alotaibi, S. S., Madkour, D. A., Orabi, S. H., Elbaz, H. T., and Ahmed, M. M. (2021). *Moringa oleifera* ethanolic extract ameliorates testicular dysfunction resulting from high-fat diet–induced obesity in rats. *Andrologia*, 53 (8): e14126.

Al-Shebli, W. C. H., and Al-Anbari, I. H. (2023). Studying the antioxidant activity of moringa

leaf extracts (*Moringa oleifera* Lam.). *IOP Conference Series: Earth and Environmental Science*, 1262 (6): 062009.

Ambwani, S., Chand, P., Kaur, A., Semwal, S., Verma, J., and Ambwani, T. K. (2024). Unraveling the Medicinal Properties of *Tinospora cordifolia* in Chicken Lymphocytes: Antioxidant and Immunomodulatory Perspectives. *Journal of Scientific Research and Reports*, 30(12): 282-297.

Ambwani, S., Chand, P., Kumawat, S. K., Singh, H., and Koranga, D. (2025). *In vitro* Analysis of Antioxidant Capacity of *Bauhinia variegata* (L.) Benth Leaf Extract. *Pharmacological Research - Natural Products*, 8: 100350.

Ambwani, S., Dolma, R., Chand, P., Kaur A., and Ambwani, T. K. (2026). Phytochemical analysis of *Andrographis paniculata* (Burm.f.) Nees leaf extract and *in vitro* assessment of its antioxidant, anti-inflammatory and immunomodulating properties. *Pharmacological Research - Natural Products*, 11: 100612.

Ambwani, S., Dolma, R., Sharma, R., Kaur, A., Singh, H., Ruj, A., and Ambwani, T. K. (2023). Modulation of inflammatory and oxidative stress biomarkers due to dexamethasone exposure in chicken splenocytes. *Veterinary Immunology and Immunopathology*, 262: 110632.

Ambwani, S., Tandon, R., and Ambwani, T. K. (2019). Metal Nano Delivery Systems for Improved Efficacy of Herbal Drugs. *Biosciences Biotechnology Research Asia*,16(2): 251-261.

Azeem, M., Pirjan, K., Qasim, M., Mahmood, A., Javed, T., Muhammad, H., and Rahimi, M. (2023). Salinity stress improves antioxidant potential by modulating physio-biochemical responses in *Moringa oleifera* Lam. *Scientific Reports*, 13 (1): 2895.

Badgujar, P. C., Pawar, N. N., Chandratre, G. A., Telang, A. G., and Sharma, A. K. (2015). Fipronil-induced oxidative stress in kidney and brain of mice: Protective effect of vitamin E and vitamin C. *Pesticide Biochemistry and Physiology*, 118: 10-18.

Bhattacharya, A., Chatterjee, A., and Ghosh, S. (2018). A review of the phytochemical and pharmacological potentials of *Moringa oleifera*. *Journal of Pharmacognosy and Phytochemistry*, 7(3): 2244-2251.

Cory, H., Passarelli, S., Szeto, J., Tamez, M., and Mattei, J. (2018). The role of polyphenols in human health and food systems: A mini-review. *Frontiers in Nutrition*, 5: 87.

Darul Raiyaan, G. I., Sankari, D., Balaji, G., and Kantha Deivi, A. (2024). Free radical scavenging and antioxidant enhancing ability of *Moringa oleifera* leaf extract against gamma radiation-induced enzyme toxicity in the liver of *Pangasius sutchi*. *Frontiers in Health Informatics*, 13 (3): 841-852.

De Leon, J. A. D., and Borges, C. R. (2020). Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. *Journal of Visualized Experiments*, 159: 10.3791/61122.

Dobutr, T., Jangpromma, N., Patramanon, R., Daduang, J., Kulchat, S., Areemit, J., Lomthaisong, K., and Daduang, S. (2025). Screening of aqueous plant extracts for immunomodulatory effects on immune cells and cytokine production: *In vitro* and *in vivo* analyses. *Heliyon*, 11(4): e42692.

Dong, A., Yu, J., Chen, X., and Wang, L.-S. (2021). Potential of dietary supplementation with berries to enhance immunity in humans: A mini-review. *Journal of Food Bioactives*, 16: 19-24.

Duraisamy, R., Oycha, O. A., and Hirpaye, B. Y. (2024). Response surface methodology optimization for extraction of antioxidants from *Moringa stenopetala* leaf, phytochemical screening, and DPPH antioxidant assay. *Journal of Chemistry*, 1: 6572252.

Eladia, R. E., and Ampode, K. M. B. (2021). *Moringa oleifera* pod meal: Nutrient

analysis and its effect on growth performance and cell-mediated immunity of broiler chickens. *Journal of Animal Health and Production*, 9(2): 170-177.

El-Sherbiny, G. M., Alluqmani, A. J., Elsehemy, I. A., and Kalaba, M. H. (2024). Antibacterial, antioxidant, cytotoxicity, and phytochemical screening of *Moringa oleifera* leaves. *Scientific Reports*, 14 (1): 30485.

González-Romero, J., Arranz-Arranz, S., Verardo, V., García-Villanova, B. and Guerra-Hernández, E. J. (2020). Bioactive compounds and antioxidant capacity of Moringa leaves grown in Spain versus 28 leaves commonly consumed in pre-packaged salads. *Processes*, 8(10): 1297.

Hamdy, N. M. (2023). Effect of *Moringa oleifera* Lam. leaf extract in treating pneumonia. *Egyptian Journal of Desert Research*, 73(2): 423-442.

Kaleo, I. V., Gao, Q., Liu, B., Sun, C., Zhou, Q., Zhang, H. and Song, C. (2019). Effects of *Moringa oleifera* leaf extract on growth performance, physiological and immune response, and related immune gene expression of *Macrobrachium rosenbergii* with *Vibrio anguillarum* and ammonia stress. *Fish & Shellfish Immunology*, 89: 603-613.

Kashyap, P., Kumar, S., Riar, C. S., Jindal, N., Baniwal, P., Guiné, R. P., and Kumar, H. (2022). Recent advances in drumstick (*Moringa oleifera*) leaves bioactive compounds: Composition, health benefits, bioaccessibility, and dietary applications. *Antioxidants*, 11(2): 402.

Kumawat M, and Une H. (2024). Effect of *Lactobacillus acidophilus*, Calcium, and *Moringa oleifera* leaves extract co-administration can prevent chemical-induced carcinogenesis. *Arab Journal of Gastroenterology*, 25(4): 421-436.

Mir, M. A., Sawhney, S. S., and Jassal, M. M. S. (2013). Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. *Wudpecker Journal of Pharmacy and Pharmacology*, 2(1): 1-5.

Mohamed, M. A., El-Mleeh, A. A., Hamad, R. T., Abu-Alya, I. S., El-Hewaity, M. H., Elbestawy, A. R., and Elshabrawy, O. I. (2023). Immunostimulant potential of *Moringa oleifera* leaves alcoholic extract versus oregano essential oil against cyclophosphamide-induced immunosuppression in broiler chicks. *Tropical Animal Health and Production*, 55(3): 209.

Mohlala, K., Offor, U., Monageng, E., Takalani, N. B., and Opuwari, C. S. (2023). Overview of the effects of *Moringa oleifera* leaf extract on oxidative stress and male infertility: a review. *Applied Sciences*, 13(7): 4387.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1–2): 55-63.

Nazim, M., Raza, W., ul Hassan Nasim, F., Anjum, S., Ullah, H., Nawaz, A., Ayaz, M., and Daglia, M. (2025). *Moringa oleifera*: a comprehensive review with special emphasis on phytochemistry. *Phytochemistry Reviews,* 25: 891-944.

Okwu, D. E., and Iroabuchi, F. (2009). Phytochemical composition and biological activities of *Uvaria chamae* and *Clerodendron splendens*. *E-Journal of Chemistry*, 6(2): 553-560.

Opuwari, C. S., Matshipi, M. N., Phaahla, M. K., Setumo, M. A., Moraswi, R. T., Zitha, A. A., and Choma, S. S. (2020). Androgenic effect of aqueous leaf extract of *Moringa oleifera* on Leydig TM3 cells *in vitro*. *Andrologia*, 52(11): e13825.

Osman, S. O. M., Paliwal, L., Ambwani, S., and Ambwani, T. K. (2024). Comparative analysis of *Albizia lebbeck* and *Lawsonia inermis*: Phytochemical estimation and their immunomodulatory effects. *The Journal of Phytopharmacology*, 13(6):464-469.

Oyewo, E. B., Akanji, M. A., and Adekunle, A. S. (2012). Immunomodulation capabilities of aqueous leaf extract of *Phyllanthus amarus*

in male Wistar rats. *Report and Opinion*, 4(1): 22-37.

Pandey, Y., and Ambwani, S. (2022). Nano Metal based Herbal theranostics for Cancer management: coalescing nature's boon with nanotechnological advancement. *Current Pharmaceutical Biotechnology*, 23(1): 30-46.

Pareek, A., Pant, M., Gupta, M. M., Kashania, P., Ratan, Y., Jain, V., and Chuturgoon, A. A. (2023). *Moringa oleifera*: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical, clinical, phytochemical, and toxicological aspects. *International Journal of Molecular Sciences*, 24(3): 2098.

Rose, N. R., and Friedman, H. (1976). Manual of Clinical Immunology. *American Society for Microbiology Press*, Washington, DC. Pp 660-664.

Saini, R. K., Sivanesan, I., and Keum, Y. S. (2016). Phytochemicals of *Moringa oleifera*: Nutritional, therapeutic, and industrial significance. *3 Biotech*, 6 (2): 203.

Saleem, A., Saleem, M., and Akhtar, M. F. (2020). Antioxidant, anti-inflammatory, and antiarthritic potential of *Moringa oleifera* Lam.: An ethnomedicinal plant of the family Moringaceae. *South African Journal of Botany*, 128: 246-256.

Salvi, M., Battaglia, V., Brunati, A. M., La Rocca, N., Tibaldi, E., Pietrangeli, P., Marcocci, L., Mondovì, B., Rossi, C. A., and Toninello, A. (2007). Catalase takes part in rat liver mitochondria oxidative stress defense. *Journal of Biological Chemistry*, 282(33): 24407-24415.

Siddiqui, N., Rauf, A., Latif, A., and Mahmood, Z. (2017). Spectrophotometric determination of total phenolic content and spectral studies of the herbal Unani drug Gul-e-Zoofa (*Nepeta bracteata* Benth). *Journal of Taibah University Medical Sciences*, 12(4): 360-363.

Singh, D., Arya, P. V., Aggarwal, V. P., and Gupta, R. S. (2014). Evaluation of antioxidant and hepatoprotective activities of *Moringa oleifera* Lam. leaves in carbon tetrachloride-intoxicated rats. *Antioxidants*, 3(3): 569-591.

Singh, D., Singh, R., Singh, P. and Gupta, R. S. (2009). Effects of embelin on lipid peroxidation and free radical scavenging activity against liver damage in rats. *Basic & Clinical Pharmacology & Toxicology*, 105(4): 243-248.

Sultana, S. (2020). Nutritional and functional properties of *Moringa oleifera*. *Metabolism Open*, 8: 100061.

Wang, Z., Yang, S., Gao, Y., and Huang, J. (2022). Extraction and purification of antioxidative flavonoids from *Chionanthus retusa* leaf. *Frontiers in Bioengineering and Biotechnology*, 10:1085562.

Received: January 02, 2026
Accepted: March 30, 2026