

IN VITRO INVESTIGATION OF MORINGA OLEIFERA LEAVES FOR ANTI-HYPERLIPIDIMIC ACTIVITY

Dr. Malepati Sandhya Rani*, E. Aman Afrin, G. Lahithya, G. Meena, D. Madhavi, G. Lal Bee

Sri Lakshmi Venkateswara Institute of Pharmaceutical Sciences, Kothapeta, Proddatur, Kadapa, A.P. Department of Pharmacognosy.

Article Received: 16 January 2026 | Article Revised: 6 February 2026 | Article Accepted: 26 February 2026

*Corresponding Author: Dr. Malepati Sandhya Rani

Sri Lakshmi Venkateswara Institute of Pharmaceutical Sciences, Kothapeta, Proddatur, Kadapa, A.P. Department of Pharmacognosy.

DOI: <https://doi.org/10.5281/zenodo.18831477>

How to cite this Article: Dr. Malepati Sandhya Rani, E. Aman Afrin G. Lahithya G. Meena D. Madhavi G. Lal Bee (2026) IN VITRO INVESTIGATION OF MORINGA OLEIFERA LEAVES FOR ANTI-HYPERLIPIDIMIC ACTIVITY. World Journal of Pharmaceutical Science and Research, 5(3), 278-284. <https://doi.org/10.5281/zenodo.18831477>



Copyright © 2026 Dr. Malepati Sandhya Rani | World Journal of Pharmaceutical Science and Research.

This work is licensed under creative Commons Attribution-NonCommercial 4.0 International license (CC BY-NC 4.0).

ABSTRACT

Hyperlipidaemia is a major metabolic disorder characterized by elevated cholesterol and lipid levels in the blood, leading to cardiovascular diseases such as atherosclerosis and coronary artery disease. Although synthetic lipid-lowering agents are effective, their long-term use may cause adverse effects, necessitating safer alternatives. The present study aimed to evaluate the in vitro anti-hyperlipidaemic activity of methanolic extract of Moringa oleifera leaves. Leaves were shade-dried, powdered, and extracted by maceration using methanol. Preliminary phytochemical screening was performed to identify bioactive constituents. Anti-hyperlipidaemic activity was assessed using an in vitro cholesterol-binding assay at concentrations ranging from 50 to 300 micrograms per milliliter. Cholestyramine was used as the standard drug. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides, phenols, and terpenoids. The extract exhibited concentration-dependent cholesterol-binding activity, showing 15.29 percent inhibition at 50 micrograms per milliliter and 62.35 percent at 300 micrograms per milliliter. The standard drug demonstrated 72.24 percent inhibition at 300 micrograms per milliliter. The results indicate that Moringa oleifera possesses significant lipid-lowering potential and may serve as a natural therapeutic agent for the management of hyperlipidaemia. Further in vivo and clinical studies are recommended to confirm its efficacy and safety.

KEYWORDS: Moringa oleifera; Hyperlipidemia; Cholesterol binding assay; Phytochemical screening; Methanolic extract.

INTRODUCTION

Hyperlipidaemia is a metabolic disorder characterized by elevated levels of total cholesterol, low-density lipoprotein cholesterol, triglycerides, or reduced high-density lipoprotein cholesterol in the bloodstream.^[1,2] It is a major risk factor

for cardiovascular diseases, including coronary artery disease, myocardial infarction, and stroke.^[3] The increasing prevalence of hyperlipidaemia worldwide has become a significant public health concern.^[4]

Although synthetic hypolipidaemic drugs such as statins and bile acid sequestrants are widely prescribed, prolonged use may lead to adverse effects including hepatotoxicity, gastrointestinal disturbances, and muscle-related complications.^[5,6] Therefore, there is growing interest in plant-based alternatives with fewer side effects.^[7]

Moringa oleifera, commonly known as drumstick tree, is widely distributed in tropical and subtropical regions.^[8] It is rich in bioactive compounds such as flavonoids, phenolic acids, saponins, tannins, and alkaloids.^[9] Several studies have reported its antioxidant, anti-inflammatory, antidiabetic, and cardioprotective activities.^[10] However, scientific evaluation of its anti-hyperlipidaemic activity through in vitro methods remains essential.^[11, 12]

The present study was undertaken to evaluate the in vitro anti-hyperlipidaemic activity of methanolic extract of *Moringa oleifera* leaves using a cholesterol-binding assay and to correlate the activity with its phytochemical constituents.

MATERIALS AND METHODS

The present study was an experimental laboratory-based investigation designed to evaluate the phytochemical constituents and in vitro antihyperlipidaemic activity of the methanolic extract of *Moringa oleifera* leaves using a cholesterol-binding assay.

Collection and Authentication of Plant Material

Fresh leaves of *Moringa oleifera* were collected from a local area in Andhra Pradesh, India. The plant material was washed thoroughly with distilled water to remove dust and impurities. The leaves were shade-dried at room temperature for 7–10 days and then coarsely powdered using a mechanical grinder. The powdered material was stored in an airtight container until further use.



Figure 1: Moringa oleifera leaves and powder Preparation of Methanolic Extract.

The dried powdered leaves were subjected to extraction using methanol as a solvent by the maceration method. Approximately 100 g of powdered plant material was soaked in 500 mL of methanol in a clean glass container and kept for 72 hours at room temperature with occasional shaking. The mixture was then filtered using Whatman No. 1 filter

paper. The filtrate was concentrated under reduced pressure using a rotary evaporator to obtain a semi-solid extract. The extract was dried, weighed, and stored in a refrigerator at 4°C until further analysis.

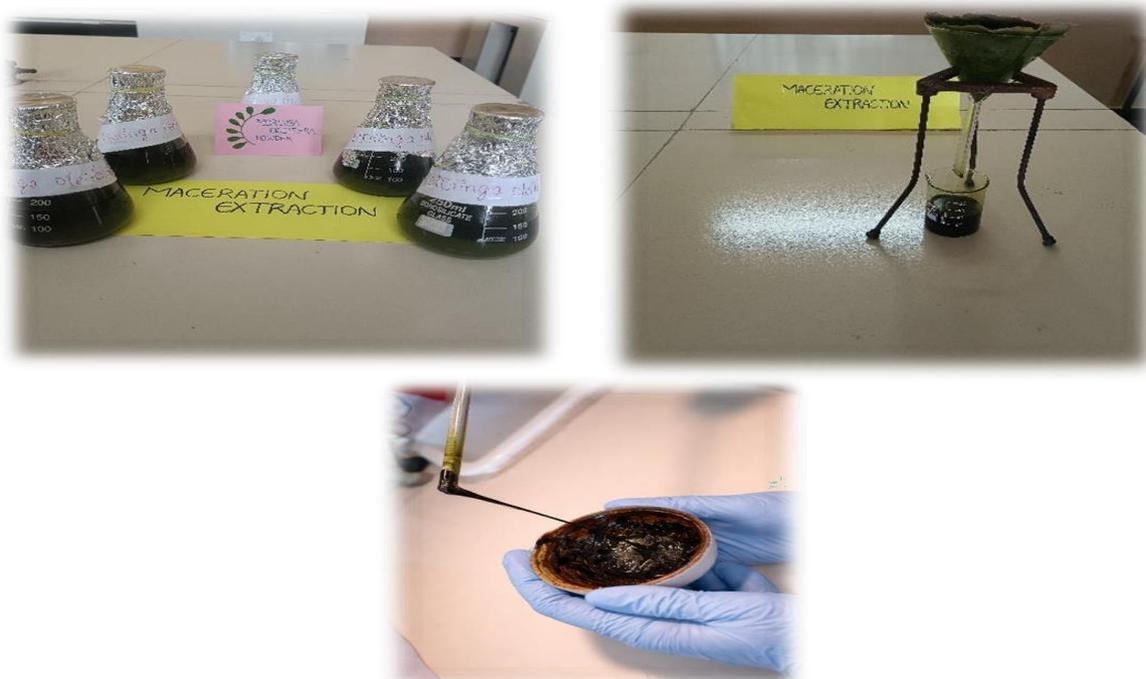


Figure 2: Maceration process, filtration and crude product of moringa powder.

Preliminary Phytochemical Screening

The methanolic extract was subjected to qualitative phytochemical screening to detect the presence of various secondary metabolites Alkaloids, Flavonoids, Tannins, Saponins, Phenols, Glycosides, Terpenoids, Steroids, Fixed oils using standard procedures.

The appearance of characteristic color changes or precipitate formation indicated the presence of respective phytoconstituents.

In Vitro Cholesterol-Binding Assay

The antihyperlipidaemic activity of the methanolic extract was evaluated using an in vitro cholesterol- binding assay.

Preparation of Test Solutions

The extract was dissolved in methanol to prepare different concentrations of 50, 100, 150, 200, 250, and 300 µg/mL. Cholestyramine was used as the standard drug for comparison and prepared in similar concentrations.

Assay Procedure

A known concentration of cholesterol solution was prepared and mixed with various concentrations of the extract. The reaction mixture was incubated at 37°C for a specified period to allow interaction between cholesterol and the extract. After incubation, the absorbance of the solution was measured using a UV–Visible spectrophotometer at the appropriate wavelength against a blank. The reduction in absorbance indicated cholesterol- binding activity.

Calculation of Percentage Inhibition

The percentage inhibition of cholesterol was calculated using the following formula: Percentage inhibition equals Control absorbance minus Sample absorbance divided by Control absorbance multiplied by 100.

Statistical Analysis: All experiments were performed in triplicate, and results were expressed as mean percentage inhibition.

Table 1: Preliminary phytochemical screening of methanolic extract of moringa oleifera.

S.NO	TEST FOR	METHANOLIC EXTRACT
1.	Alkaloids	Positive
2.	Flavonoids	Positive
3.	Tannins	Positive
4.	Saponins	Positive
5.	Glycosides	Positive
6.	Phenols	Positive
7.	Terpinols	Positive



Figure-3: Phytochemical screening of moringa oleifera.

Table 2: Anti hyperlipidemic Activity

S. No	Sample Solution (mL)	Concentration (µg/mL)	Sample Absorbance (530 nm)	% Cholesterol Binding of Sample	Cholestyramine Absorbance (530 nm)	% Cholesterol Binding of Standard
1	1	50	0.72	15.29 %	0.48	27.27 %
2	2	100	0.63	25.88 %	0.41	37.87 %
3	3	150	0.55	35.29 %	0.35	46.96 %
4	4	200	0.47	44.70 %	0.28	57.57 %
5	5	250	0.39	54.11 %	0.22	66.66 %
6	6	300	0.32	62.35 %	0.17	72.24 %
Blank	–	0	0.85	–	0.66	–

CALCULATION

$$\% \text{Inhibition} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

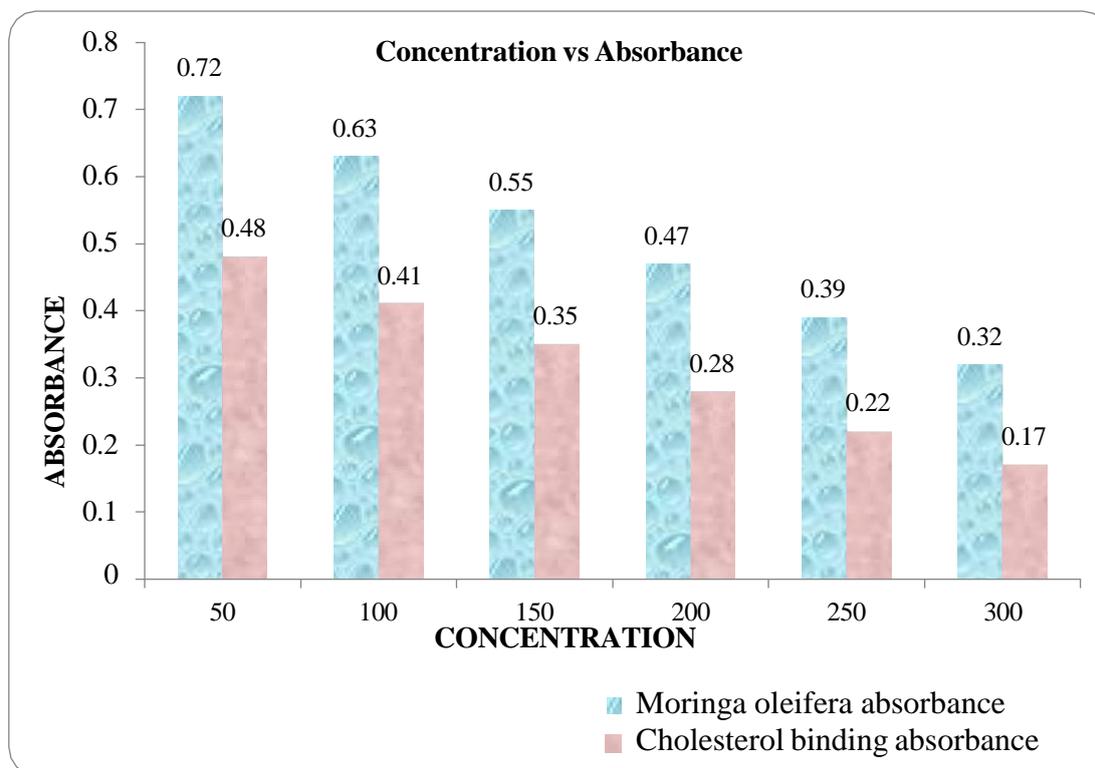


Figure-4: Concentration vs Absorbance.

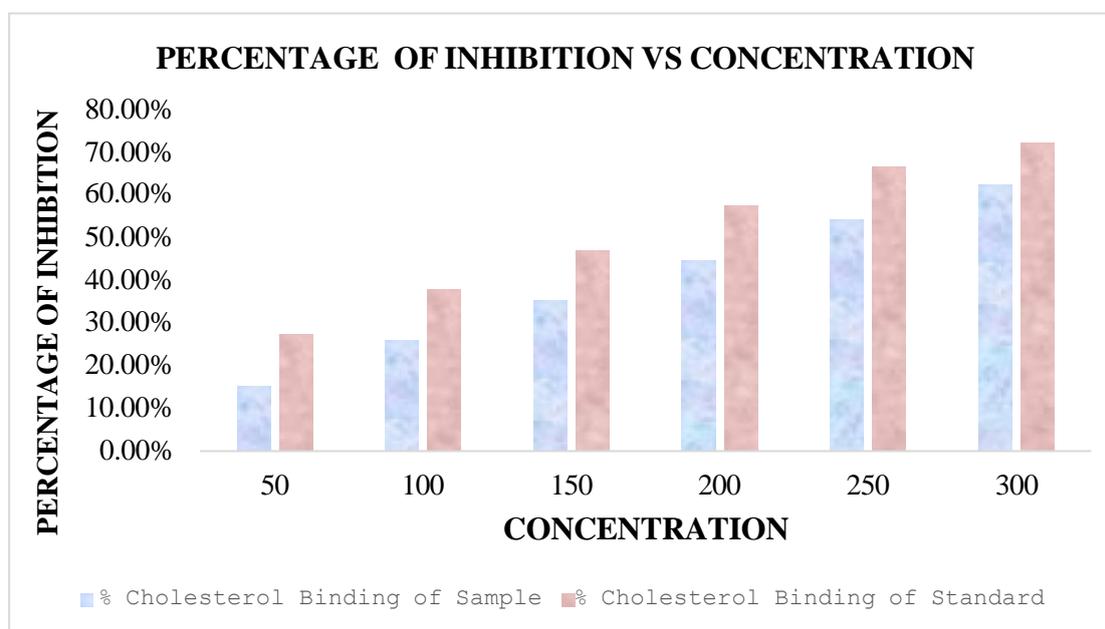


Figure 5: Percentage of inhibition vs Concentration.

DISCUSSION

The present investigation evaluated the in vitro anti-Hyperlipidemic activity of the methanolic extract of Moringa oleifera leaves using a cholesterol-binding assay. The results demonstrated a clear concentration-dependent increase in

cholesterol-binding activity. The extract exhibited 15.29% inhibition at 50 µg/mL, which progressively increased to 62.35% at 300 µg/mL. Although the standard drug cholestyramine showed comparatively higher inhibition (72.24% at 300 µg/mL), the plant extract displayed significant activity, indicating promising lipid-lowering potential.

The cholesterol-binding assay is an important preliminary screening method that evaluates the ability of compounds to bind free cholesterol, thereby reducing its intestinal absorption. The observed inhibitory activity suggests that the extract may reduce circulating cholesterol levels by interfering with cholesterol solubility and absorption mechanisms.

The phytochemical screening revealed the presence of flavonoids, saponins, tannins, phenols, alkaloids, glycosides, and terpenoids. These bioactive constituents are well known for their hypolipidaemic effects. Saponins are particularly recognized for their ability to bind bile acids and cholesterol in the gastrointestinal tract, forming insoluble complexes that are excreted from the body. This process enhances bile acid elimination and stimulates hepatic conversion of cholesterol into bile acids, thereby lowering serum cholesterol levels.

Flavonoids and phenolic compounds contribute through antioxidant mechanisms. Oxidative stress plays a major role in the development of hyperlipidemia and atherosclerosis by promoting lipid peroxidation and low-density lipoprotein oxidation. The antioxidant property of flavonoids may protect lipoproteins from oxidative modification, reducing the progression of atherosclerotic plaque formation. Furthermore, phenolic compounds may regulate lipid metabolism by influencing key enzymes such as HMG-CoA reductase, which is involved in cholesterol biosynthesis.

The concentration-dependent response observed in this study indicates a dose-related pharmacological effect, suggesting that higher concentrations contain sufficient active phytoconstituents to exert stronger cholesterol-binding action. The gradual increase in inhibition percentage from 15.29% to 62.35% supports the reliability and reproducibility of the assay.

Although the extract showed slightly lower activity compared to cholestyramine, it is important to note that synthetic bile acid sequestrants may cause gastrointestinal side effects such as constipation, bloating, and interference with fat-soluble vitamin absorption. In contrast, plant-based agents like *Moringa oleifera* may offer safer long-term therapeutic alternatives with additional antioxidant and cardioprotective benefits.

The findings of the present study are consistent with previous reports suggesting hypolipidaemic and cardioprotective properties of *Moringa oleifera*. Earlier *in vivo* studies have demonstrated reductions in total cholesterol, triglycerides, and low-density lipoprotein levels following administration of *Moringa* extracts. The present *in vitro* data support these findings and provide mechanistic insight into its cholesterol-binding potential.

However, certain limitations must be acknowledged. The study was restricted to *in vitro* evaluation, which does not fully replicate complex physiological conditions such as intestinal absorption, hepatic metabolism, and systemic lipid regulation. The bioavailability of active phytoconstituents and their metabolic transformation *in vivo* were not assessed. Additionally, the exact active compound responsible for the observed activity was not isolated or quantified.

CONCLUSION

The present study demonstrates that the methanolic extract of *Moringa oleifera* leaves exhibits significant concentration-dependent *in vitro* cholesterol-binding activity, reaching 62.35% inhibition at 300 µg/mL. The lipid-

lowering potential may be attributed to the presence of saponins, flavonoids, and phenolic compounds. Although slightly lower than the standard drug, the extract shows promising natural therapeutic potential. Further in vivo and clinical investigations are required to confirm its efficacy and safety for the management of hyperlipidaemia.

ACKNOWLEDGEMENTS

The authors acknowledge Sri Lakshmi Venkateshwara Institute of Pharmaceutical Sciences for providing necessary facilities for conducting this research.

Conflict of Interest

The authors declare no conflict of interest

REFERENCES

1. Grundy SM. Hyperlipidemia, atherosclerosis and cardiovascular disease. *American Journal of Clinical Nutrition*, 2006; 83(6): 1453–1463. <https://doi.org/10.1093/ajcn/83.6.1453>
2. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales NP, Phivthong-Ngam L, Ratanachamnong P, Srisawat S, Pongrapeeporn KU. The in vitro and in vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of *Moringa oleifera* leaf extract. *Journal of Ethnopharmacology*, 2008; 116(3): 439–446. <https://doi.org/10.1016/j.jep.2007.12.010>
3. Fahey JW. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. *Trees for Life Journal*, 2005; 1(5): 1–15.
4. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytotherapy Research*, 2007; 21(1): 17–25. <https://doi.org/10.1002/ptr.2023>
5. Mbikay M. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia: A review. *Frontiers in Pharmacology*, 2012; 3: 24. <https://doi.org/10.3389/fphar.2012.00024>
6. Ghasi S, Nwobodo E, Ofili JO. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* in high-fat diet fed Wistar rats. *Journal of Ethnopharmacology*, 2000; 69(1): 21–25. [https://doi.org/10.1016/S0378-8741\(99\)00106-3](https://doi.org/10.1016/S0378-8741(99)00106-3)
7. Mehta LK, Balaraman R, Amin AH, Bafna PA, Gulati OD. Effect of fruits of *Moringa oleifera* on the lipid profile of normal and hypercholesterolaemic rabbits. *Journal of Ethnopharmacology*, 2003; 86(2–3): 191–195. [https://doi.org/10.1016/S0378-8741\(03\)00075-8](https://doi.org/10.1016/S0378-8741(03)00075-8)
8. Trease GE, Evans WC. *Pharmacognosy*. 16th ed. London: Saunders; 2009.
9. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman and Hall; 1998.
10. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods for Human Nutrition*, 2009; 64(4): 303–311. <https://doi.org/10.1007/s11130-009-0141-0>
11. Jain PG, Patil SD, Haswani NG, Girase MV, Surana SJ. Hypolipidemic activity of *Moringa oleifera* Lam., Moringaceae, on high fat diet induced hyperlipidemia in albino rats. *Brazilian Journal of Pharmacognosy*, 2010; 20(6): 969–973. <https://doi.org/10.1590/S0102-695X2010005000035>
12. Ezzat SM, et al. Chemical constituents and biological activity of *Moringa oleifera* leaves. *Pharmaceutical Biology*, 2011; 49(12): 1292–1299 <https://doi.org/10.3109/13880209.2011.586206>