

Comparative Evaluation of Solvent Extraction on the Phytochemical Profile and Antioxidant Potential of *Moringa oleifera* leaf extracts

¹Ritu Raj Negi and ^{*2}Neetu Pandey

School of Applied Chemistry and Basic Sciences,
Sardar Bhagwan Singh University, Dehradun-248001, Uttarakhand, India

Email: neetu_bhtt@yahoo.co.in

DOI 10.51129/ujpah-2025-39-2(3)

Received- 28 October, 2025

Revised – 30 October, 2025

Accepted – 03 October, 2025

Published- 27 December, 2025

Abstract - Antioxidants are molecules that prevent or counteract the harmful effects of reactive oxygen species and free radicals, which are unstable molecules produced during metabolic processes or from external factors such as pollution, UV radiation, and smoking. These reactive molecules induce oxidative stress, resulting in cellular damage and DNA mutations, and play a role in aging, inflammation, and chronic diseases like cancer, diabetes, and cardiovascular disorders. Antioxidants mitigate this damage by donating electrons to free radicals, stabilizing them and preventing further oxidative damage chain reactions. Antioxidants sourced from plants, especially fruits, vegetables, and herbs, are increasingly recognized for their natural effectiveness and possible health advantages. *Moringa oleifera* is known for its numerous pharmacological attributes, with antioxidant activity being prominent. In this study, the antioxidant properties of *Moringa oleifera* were studied and evaluated using DPPH Scavenging Activity. IC5050 value for different solvents i.e., 33.96 µg/mL for methanol, 41.7 µg/mL for ethanol, and 146.9 µg/mL for chloroform, which

indicated ethanolic extract showed good scavenging activity. The results were found to be aligned with previous studies on the antioxidant properties of *Moringa oleifera*.

Keywords: Antioxidants, DPPH Scavenging Activity, Free radical, Ascorbic acid

Introduction

Moringa oleifera is a tropical tree indigenous to the lower parts of the Himalaya mountain range. It is now spread throughout tropical regions in Africa, Asia, and Southern-America^[1]. The tree is one of the 13 known species of the single genus *Moringaceae* family, classified in the order of *Brassicales*^[2] and has earned the name ‘miracle’ tree due to its versatile properties.

The bark, sap, roots, leaves, seeds, and flowers are used in traditional medicine^[3-4]. Research has examined how it might affect blood lipid profiles and insulin secretion^[5]. Extracts from leaves contain various polyphenols, which are under basic research to determine their potential effects in humans^[6]. Despite considerable preliminary research to determine if moringa components

have bioactive properties, no high-quality evidence has been found to indicate that it has any effect on health or diseases^[5]. Oxidative stress seems to play a significant role in many human diseases, including cancers^[7], and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases^[8], as well as inflammation and problems caused by cell and cutaneous ageing^[9]. Antioxidants play a crucial role in neutralizing free radicals, reducing oxidative stress, and preventing cellular damage linked to chronic diseases such as cardiovascular disorders, diabetes, and cancer. As a consequence of action of antioxidants against freeradicals, the cellular components are prevented from damage^[10]. *Moringa oleifera*, has well-documented antioxidant properties due to its rich bioactive compound profile, which includes vitamins, polyphenols, and flavonoids. *Moringa oleifera* leaves are rich in polyphenols and flavonoids, both of which have potent antioxidant effects. These compounds scavenge free radicals, inhibit lipid peroxidation, and enhance the body's natural antioxidant enzymes (such as catalase, superoxide dismutase, and glutathione peroxidase), which collectively protect cells from oxidative damage^[11]. *Moringaoleifera* contains high levels of Vitamin C and beta-carotene, which play a crucial role in reducing oxidative stress by scavenging free radicals.

Aim and Objective

Aim- In vitro evaluation of antioxidant potential of *Moringa oleifera* leaves.

Objective- To achieve the above aim the research project have been divided into following objectives:

1. To evaluate the antioxidant activity of *Moringa oleifera* leaves through extraction and assessment of free

radical scavenging activity using standard in vitro assays.

2. In vitro **evaluation of** the antioxidant activity of the extracts was done using as DPPH (2,2-diphenyl-1-picrylhydrazyl) assay.
3. Evaluating antioxidant activity of *M. oleifera* leaves and explore their potential in preventing or managing diseases like cancer, diabetes, and cardiovascular issues.

Material and Methodology

Collection of Plant Material

Leaves of *Moringa oleifera* were procured from the locality of Balawala, Dehradun, Uttarakhand in the month of January 2024. A selected plant was identified and authenticated by the Botanical Survey of India, Dehradun and voucher specimen was deposited at the herbarium. The leaves were shade dried for few days until there was no moisture left in them then crushed by hands for further extraction.

Extraction

An orbital shaker was used for extraction preparation. Dry grounded *Moringa* leaves were mixed with 200 mL of different solvents i.e., ethanol, methanol, chloroform and petroleum ether and placed on the shaker for 24 hrs at 100 rpm. Samples were then centrifuged (MPW®-352R, Med. Instruments) at 5,000 rpm for 15 min. The supernatant of the samples was again filtered with Whatman filter paper, and the filtrates obtained were carried to a rotary evaporator for evaporation of alcohol under reduced pressure at 50°C, 120 rpm. The extracts obtained were stored at 4°C until further analysis^[12].

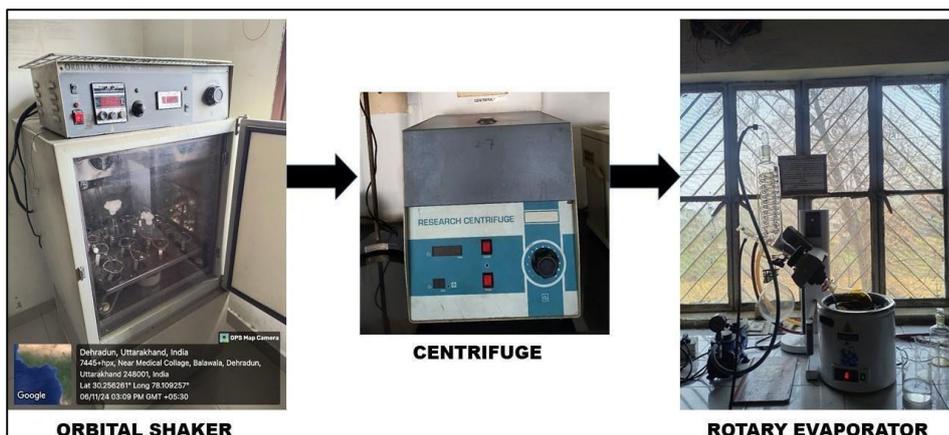


Figure-1 Instrumentation of extraction process

DPPH Spectrophotometric assay

Series of dilutions of the methanolic extracts ranging from 20-100µg/ml was prepared from stock solution (1mg/mL). A measure of 1 ml of the extract was mixed with 3 ml of 0.3 mg/ml of DPPH radical. The mixture was shaken vigorously and allowed to stand for 30 minutes at room temperature under dark conditions. The absorbance of the mixture was read at 517 nm using UV-Vis spectrophotometer. The absorbance

of the resulting solution was converted into a percentage of antioxidant activity (% inhibition) by the use of the following formula:

$$\% \text{ Inhibition} = (A_0 - A_1) / A_0 \times 100$$

Where A₀ = Absorbance of the control solution containing only DPPH solution; A₁ = Absorbance in the presence of extract in DPPH solution. Ascorbic acid was used as a reference standard. The IC₅₀ values were determined from extrapolation curves of the antioxidant activity of extracts on DPPH^[13].

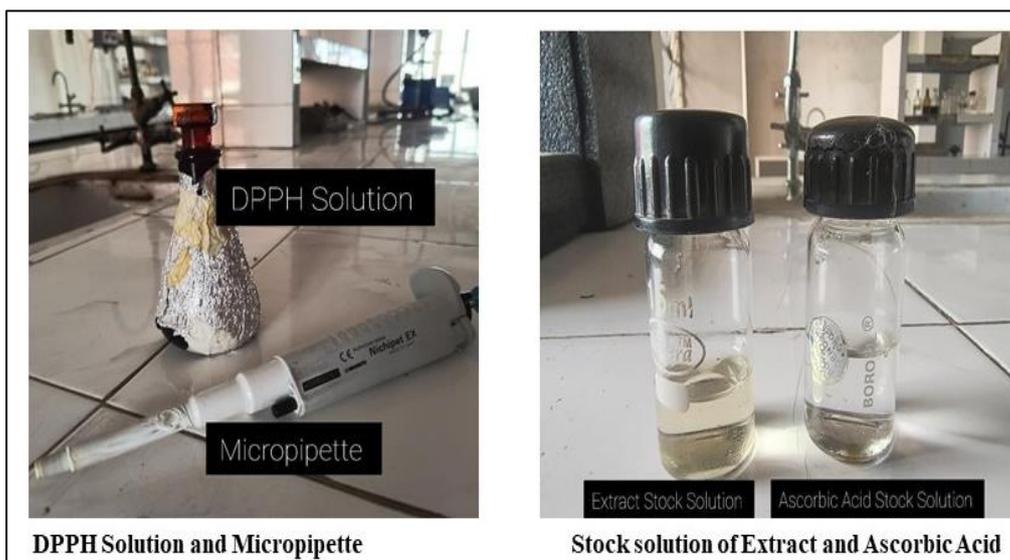


Figure-2 DPPH solution and stock solution

Phytochemical Screening

Qualitative phytochemical analysis of the crude extracts was performed using standard protocols to detect the

presence of tannins, saponins, flavonoids, steroids, terpenoids, and alkaloids.

Result and Discussion

Extraction Yield

The percentage yield of the extracts increased with solvent polarity:

chloroform (5.75%), methanol (9.63%), and ethanol (12.7%).

Table-1 Percentage yield of extracts

Extract	Percentage yield
Chloroform	5.75%
Methanol	9.63%
Ethanol	12.7%

Phytochemicals analysis

The qualitative phytochemical screening (**Table-1**) indicated that methanol and ethanol extracts contained a wider range of phyto-constituents, including tannins, saponins, flavonoids, and alkaloids. The chloroform extract showed a more limited profile.

dependent DPPH radical

scavenging activity (**Table-2**). The methanolic extract exhibited the highest activity (89.1%) inhibition at 100 µg/ mL, followed by the ethanolic (82.4%) and chloroform (39.2%) extracts. The IC50 values (**Table-3**) confirmed the superior efficacy of the methanolic extract (IC50 = 33.96 µg/ mL), which was close to that of the standard ascorbic acid (IC50 = 24.2 µg/ mL).

Determination of antioxidant activity

All extracts demonstrated concentration

Table-2 Qualitative analysis of different extracts of *M. oleifera* leaves

Constituents	Chloroform extract	Methanol extract	Ethanol extract
Tannins	+	+	-
Saponins	+	+	+
Steroids	-	-	-
Flavonoids	-	+	+
Terpenoids	-	-	+
Alkaloids	+	+	+

Table-3 The absorbance of standard solution for various concentrations

Ascorbic acid (Dilution)	% Inhibition
1µg/mL	16.96
5µg/mL	25.03
10µg/mL	31.25
15µg/mL	39.83
20µg/mL	45.36

Table-4 Absorbance of crude extracts

Dilution	% Inhibition		
	Chloroform	Ethanol	Methanol
20µg/mL	12.4	28.9	35.4
40µg/mL	21.9	48.6	57.8
60µg/mL	29.7	65.3	73.2
80µg/mL	34.5	76.8	84.6
100µg/mL	39.2	82.4	89.1

Table-5 The IC₅₀ values of Standard solution and Solvent solution

Samples	IC ₅₀ value (µg/mL)
Ascorbic acid	24.2
Methanol	33.96
Ethanol	41.7
Chloroform	146.9

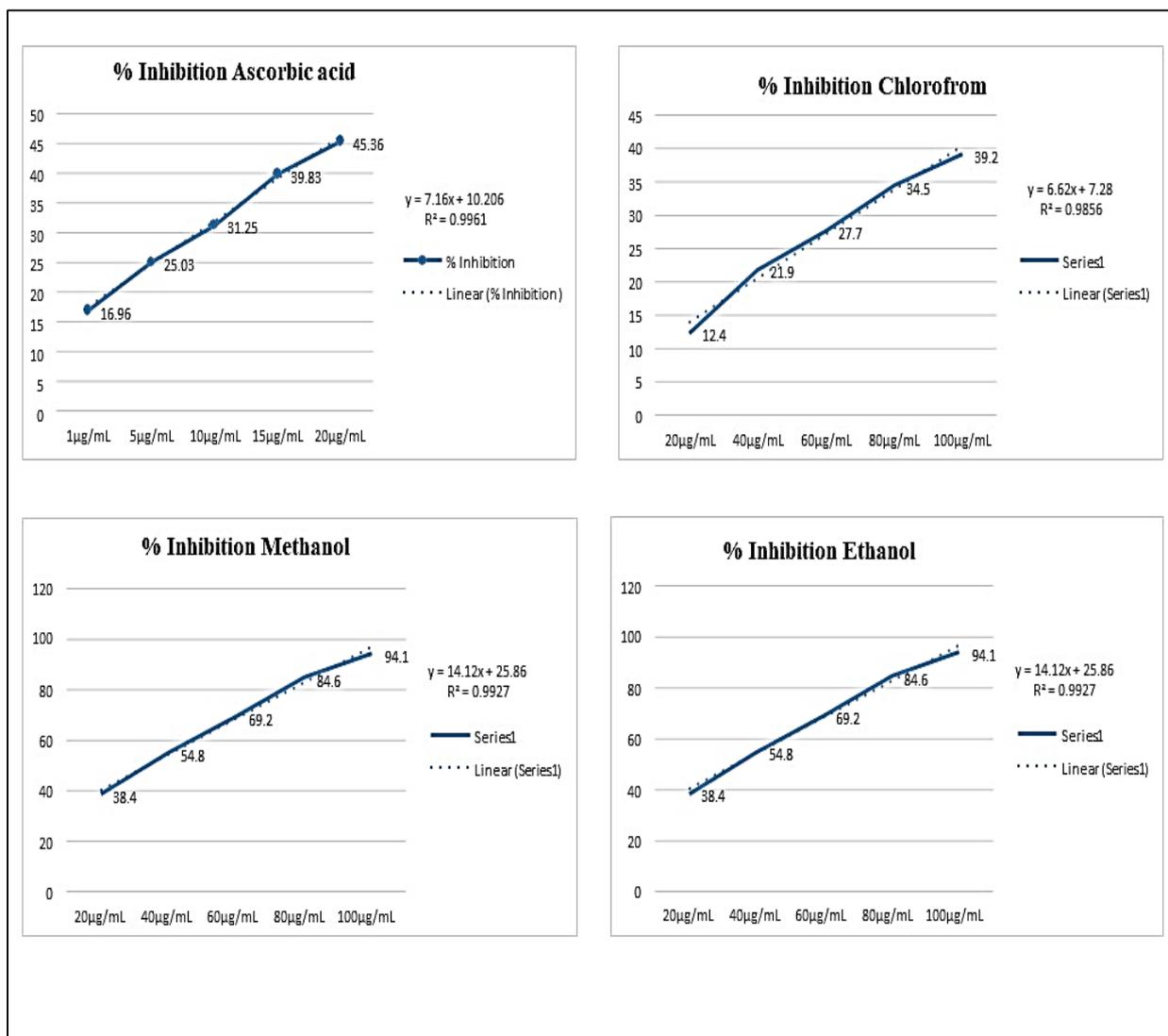


Figure-3 % RSA Graphs of Various Extract of *Moringa oleifera* leaves

According to the results, the solvent extracts of *Moringa oleifera* showed a very good DPPH radical scavenging activity. Ascorbic acid was used as a positive control which showed a maximum percentage of free radical scavenging activity i.e., 85% at a concentration of 5.0mg/ml. Also, this free radical scavenging activity (%inhibition) increased with increasing concentration of the crude extracts.

Discussion

In the present study, we have investigated the different fractions of *M. oleifera* leaves for in vitro antioxidant activity. The results demonstrate that solvent polarity critically influences the extraction efficiency of antioxidants from *Moringa oleifera* leaves. Methanol, a moderately polar solvent, out-performed other solvents such as chloroform and methanol in isolating phenolic and flavonoid compounds, which are strongly associated with free radical scavenging activity. This aligns with studies showing that methanol effectively disrupts plant cell membranes and solubilizes glycosylated phenolics, such as quercetin-3-glucoside and chlorogenic acid, which dominate *Moringa*'s antioxidant profile^[14]. Ethanol, though slightly less efficient, remains advantageous for food and pharmaceutical applications due to its lower toxicity^[15].

Methanol extracted *Moringa oleifera* leaves demonstrated the highest

free radical scavenging activity, achieving 89.1 % inhibition of DPPH radicals at a concentration of 100 µg/mL. This was comparable to the reference standard ascorbic acid (45.36 % inhibition at 20µg/mL). Ethanol extracts followed closely with 82.4% inhibition, non-polar solvents like chloroform exhibited negligible scavenging capacity (<10% inhibition), indicating that polar solvents are more effective in isolating radical neutralizing compounds.

Conclusion

The investigation into the antioxidant potential of *Moringa oleifera* leaf extracts, as evaluated through the DPPH radical scavenging assay, underscores the plant's remarkable capacity to neutralize free radicals, positioning it as a valuable natural source of antioxidants. Methanolic extracts emerged as the most efficacious, exhibiting an IC₅₀ value of 33.96 µg/mL, a performance closely rivaling the reference antioxidant ascorbic acid (IC₅₀ = 24.2 µg/mL). This robust activity can be attributed to the high concentration of polar bioactive compounds, such as glycosylated flavonoids and ascorbic acid, which are efficiently extracted by methanol due to its intermediate polarity and ability to disrupt plant cell matrices. Ethanolic extracts, while slightly less potent (IC₅₀ = 41.7 µg/mL), demonstrated significant radical scavenging activity, making them a safer and industrially viable alternative for food and

pharmaceutical applications. The low activity of chloroform extracts (IC₅₀ = 146.9 µg/mL) highlights the limited solubility of hydrophilic antioxidants in non-polar solvents. These findings emphasize the critical role of solvent selection in optimizing antioxidant recovery, with methanol and ethanol being ideal for research and commercial purposes, respectively.

The study advocates for the integration of *Moringa oleifera* leaf extracts into functional foods, nutraceuticals, and natural preservatives, where their radical scavenging properties could mitigate oxidative deterioration and enhance product shelf life. Future research should focus on in vivo validation of these findings, particularly the bio-availability and metabolic fate of *Moringa* antioxidants, as well as the development of eco-friendly extraction methods to align with sustainable practices.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

References

1. Dubey, D. K.; Dora, J.; Kumar, A. and Gulsan, R. K. A multipurpose tree *Moringa oleifera*. *International journal of pharmaceutical and chemical sciences*; 2013;2(1):415-423.
2. Razis, A. F.; Abdull, Muhammad Din Ibrahim and Saie Brindha Kntayya. "Health benefits of *Moringa oleifera*." *Asian Pac. J. Cancer. Prev.*; 2014; 15; no.20:8571-8576.
3. "*Moringa oleifera* (horseradish tree)". Climate Action Business Incubator. 17 December 2019; Retrieved 17 May; 2020.
4. NPCS Board. Hand book on Agro Based Industries (2nd Revised). *Niir Project Consultancy Services*; 2012; pp. 66; ISBN 978-9381039120.
5. "*Moringa oleifera*", Memorial Sloan-Kettering Cancer Center. Retrieved 27 February; 2014.
6. Sreelatha, S. and Padma, P. R. "Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity". *Plant Foods for Human Nutrition*; 11 November 2009; **64** (4):303–311.doi:10.1007/s11130-009-0141-0.PMID19904611.S2CID 88 01347.
7. Serafini, M.; Bellocco, R.; Wolk, A. and Ekstrom, A. *MORINGA* Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroenterology*; 2002; 123:985-91.10.1053/gast.2002.35957.
8. Di-Matteo, V. and Esposito, E. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. *Curr. Drug Target CNS Neurol Disorders*; 2003; 2:95–107.10.2174/1568007033482959

9. Kumaran, A. and Karunakaran, R. J. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chem.*; 2006; 97:109– 14. [10.1016/j.foodchem.2005; 03:032](https://doi.org/10.1016/j.foodchem.2005.03.032).
10. Lobo, V.; Patil, A.; Phatak, A. and Chandra, N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev.*; 2010; 4(8):118–126.
11. Singh, B. N; Singh, B. R. and Singh R. L. et al. "Oxidative DNA damage protective activity, antioxidant and anti-quorum sensing potentials of *Moringa oleifera*." 2009.
12. Sultana, B.; Anwar, F. and Ashraf, M. Effect of extraction solvent/ technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*; 2009; Jun. 15; 14(6):2167-80. doi: 10.3390/molecules14062167. PMID: 19553890; PMCID: PMC6254218.
13. Ghosal, P.; Kothiyal, S.; Choudhary, Alka and Saklani, S. Sonchusarvensis: Antioxidant activity, phenolic profile, and phytochemical screening. *Kariri Science - CECAPE Biology and Health Journal*; 2023; (1)1-8:10.29327/2256856.2023.1-8.
14. Adekanmi, Abideen; Adekanmi, Sherifdeen and Adekanmi, Opeyemi. Qualitative and Quantitative Phytochemical Constituents of Moringa Leaf. 2020; 4:10-17.