

Eco-Friendly Synthesis of *Moringa Oleifera* Leaves-Based Silver Nanoparticles and their Antioxidant and Anti-Inflammatory Efficacy

M. Ramanathan¹, V. Sree Janardhanan¹, Ganna Anitha², T. Ethiraj³

¹Department of Chemistry, School of Pharmacy, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India, ²Department of Pharmaceutical Chemistry, Mayor Radhakrishnan College of Pharmacy, (Affiliated to Tamil Nadu Dr. M.G.R Medical University, Chennai), Cuddalore, Tamil Nadu, India, ³Department of Chemistry, Pannai College of Pharmacy, (Affiliated to Tamil Nadu Dr. MGR Medical University, Chennai.), Dindigul, Tamil Nadu, India

Abstract

Objectives: The objective of the study was to synthesize silver nanoparticles (AgNPs) from the ethyl acetate leaf extract of *Moringa oleifera*. Using a green synthesis approach, and to evaluate their *in vitro* antioxidant and anti-inflammatory activities relevant to diabetic management. **Materials and Methods:** AgNPs were prepared by reducing silver nitrate with *M. oleifera* Leaves ethyl acetate extract (MOET). Nanoparticle (NPs) formation and stability were confirmed using ultraviolet-visible spectrophotometry, Fourier transform infrared, energy dispersive X-ray, transmission electron microscopy, Particle size analysis, and zeta potential. Antioxidant activity was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) assays, while anti-inflammatory activity was evaluated by inhibition of protein (albumin) denaturation. **Results:** MOET AgNPs showed a characteristic surface plasmon resonance peak at 480 nm, confirming NPs formation. The AgNPs exhibited enhanced DPPH radical scavenging activity (half-maximal inhibitory concentration [IC₅₀] = 141.42 mg/mL) compared with the crude extract, though lower than Vitamin C. FRAP assay indicated weak and variable reducing power. Moderate anti-inflammatory activity was observed in the protein denaturation assay (IC₅₀ = 316.02 mg/mL), compared with diclofenac sodium. **Conclusion:** Green-synthesised *M. oleifera* AgNPs possess moderate antioxidant and anti-inflammatory activities attributed to phytochemical surface capping. Although less potent than standard drugs, they show potential as supportive agents for managing oxidative stress and inflammation associated with diabetes, warranting further optimization and *in-vivo* evaluation.

Key words: Anti-inflammatory, antioxidant, characterization, *Moringa oleifera* leaves, silver nanoparticles

INTRODUCTION

Nanoparticles (NPs) have gained substantial attention due to their wide-ranging applications and therapeutic agents, particularly in wound healing, burn treatment, and surgical applications.^[1,2] Conventional physical and chemical synthesis methods often involve toxic reagents and high energy consumption, prompting the development of greener alternatives. In this context, green synthesis using plant extracts has emerged as a safe, cost-effective, and environmentally friendly approach, where plant-derived biomolecules act as natural reducing and stabilizing agents, imparting enhanced physicochemical and biological properties to

NPs.^[3,4] This biosynthetic strategy aligns with sustainable nanotechnology principles and has found applications across pharmaceuticals, biomedicine, agriculture, and industrial sectors.^[5]

Medicinal plants are particularly attractive for NPs synthesis due to their rich diversity of bioactive phytoconstituents.

Address for correspondence:

Dr. V. Sree Janardhanan, Department of Pharmaceutical Chemistry, School of Pharmacy, Sri Balaji Vidyapeeth, Deemed-to-be-University, Puducherry - 607402, India.
E-mail: vsreejana@gmail.com

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Moringa oleifera Lam. (family: Moringaceae) is a nutritionally and therapeutically important plant, widely recognised for its high content of proteins, essential amino acids, vitamins, minerals, β -carotene, and phenolic compounds distributed across its leaves, flowers, fruits, seeds, and roots.^[6,7] Extensive traditional and scientific evidence support its antidiabetic, anti-inflammatory, antioxidant, antimicrobial, antihypertensive, hepatoprotective, and anticancer activities, making it a promising candidate for green nanomaterial synthesis.^[8,9]

Silver NPs (AgNPs) exhibit promising antioxidant and anti-inflammatory activities, primarily by scavenging free radicals, reducing pro-inflammatory cytokines, and boosting anti-inflammatory ones, making them potential therapeutic agents for oxidative stress and inflammation-related diseases, though dose, synthesis method, and potential toxicity require further research for clinical use.^[10] Metal NPs, particularly AgNPs, have attracted considerable interest owing to their potent antioxidant, antibacterial, anti-inflammatory, and anticancer activities, which are attributed to their unique physicochemical characteristics and high surface reactivity.^[11,12]

In this context, the present study aimed to synthesize AgNPs using *M. oleifera* leaves extract and to evaluate their physicochemical characteristics and biological activities, with particular emphasis on antioxidant and anti-inflammatory potential in comparison with established standards.

MATERIALS AND METHODS

Chemicals

Silver nitrate (AgNO_3), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Ascorbic acid, 2,4,6-Tripyridyl-S-triazine, ferric chloride (FeCl_3), sodium acetate, acetic acid, hydrochloric acid bovine serum albumin (BSA) (fraction V), and diclofenac sodium were procured from Sigma-Aldrich (Bangalore, India). All solvents are analytical grade was obtained from Sigma-Aldrich (Bangalore, India). Double-distilled Milli-Q water was used throughout the study and experimental procedures to avoid ionic contamination and ensure reproducibility.

Preparation of *M. oleifera* L. leaf extract

M. oleifera leaf powder was sourced from a commercially available brand Sigru churana powder (Dr. LF) Pwd.100%, manufactured by Aravind Herbal Laboratory (P) Ltd., Rajapalayam, Virudhunagar, Tamil Nadu, India [Figure 1]. Approximately 100 g of the powdered material was subjected to hot percolation extraction using ethyl acetate as the solvent in a Soxhlet apparatus. The obtained extract was filtered, concentrated, and stored at 4°C until further use for NPs synthesis.

Biosynthesis of AgNPs

Green synthesis of AgNPs was performed using the ethyl acetate leaf extract of *M. oleifera* as a reducing and stabilizing agent. In a single-step reaction, 5 mL of the plant extract was added to 95 mL of 1 mM aqueous AgNO_3 solution under continuous stirring. The reaction mixture was heated at 80°C for 5 min, during which a visible color change from pale yellow to dark brown indicated the formation of AgNPs due to surface plasmon resonance (SPR). The synthesized NPs were purified by repeated centrifugation at 10,000 rpm for 15 min to remove unbound phytoconstituents. The pellet was washed with distilled water, re-centrifuged using a cooling micro centrifuge, and finally dried in an incubator. The dried AgNPs were stored in sterile airtight containers for further characterization and biological evaluation.^[3,13,14]

Characterization of AgNPs

Ultraviolet (UV)-visible spectroscopic analysis

The formation and optical properties of biosynthesized AgNPs were confirmed using UV-visible spectrophotometry. A small aliquot of the NPs suspension was analyzed using a Shimadzu UV-1800 spectrophotometer, and absorption spectra were recorded in the wavelength range of 300–700 nm. The characteristic SPR peak of AgNPs was used as confirmation of NPs formation.

Fourier transform infrared (FTIR) spectroscopy

FTIR analysis was carried out to identify the functional groups of phytochemicals involved in the reduction and stabilization of AgNPs. Spectral measurements were recorded using a Bruker FTIR spectrometer equipped with an attenuated total reflectance accessory. The spectra were collected in the mid-infrared region of 400–4000 cm^{-1} with 16 scans at a suitable resolution.^[15]

Transmission electron microscopy (TEM)

The AgNPs film was placed on carbon-coated TEM grids and analyzed by high-resolution TEM (Model-FEI-Technai T20 Twin 200 KV instrument) from FEI Company, Hillsboro, Oregon, USA (now Thermo Fisher Scientific).^[16]

Energy dispersive X-ray (EDX) analysis

Elemental composition and purity of the synthesized AgNPs were analyzed using EDX spectroscopy coupled with the SEM (Zeiss EV-18). The presence of a strong silver signal confirmed the successful synthesis of AgNPs, while additional peaks corresponded to phytochemical residues.^[17]

Particle size distribution analysis

The average particle size, size distribution, and polydispersity index (PDI) of the AgNPs were determined using a particle size analyzer based on dynamic light scattering. This analysis

provided insights into NPs homogeneity and colloidal stability.

X-ray diffraction (XRD) analysis

The crystalline nature and phase identification of the biosynthesized AgNPs were evaluated using XRD. The diffraction pattern was recorded using an X'Pert PANalytical X-ray diffractometer operated at 40 kV and 30 mA with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$). Samples were stored in airtight containers before analysis to prevent oxidation and contamination.^[18]

Antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity of the samples was evaluated using the DPPH assay following established methods with minor modifications. A methanolic DPPH solution ($6 \times 10^{-5} \text{ M}$) was freshly prepared by dissolving 7.89 mg of DPPH in 100 mL of methanol. Appropriate volumes were adjusted proportionally when larger volumes were required. Sample solutions at different concentrations were prepared, and 500 μL of each sample was transferred into Eppendorf tubes in triplicate. To each tube, 500 μL of the DPPH solution was added and mixed thoroughly. The reaction mixtures were incubated at room temperature in the dark for 30 min. After incubation, the absorbance was measured at 520 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as the positive control, while distilled water served as the negative control. A DPPH solution without any test sample was used as the blank control. The actual absorbance of each test sample was calculated by subtracting the absorbance of the corresponding sample blank (without DPPH) from the absorbance of the reaction mixture. The percentage of DPPH radical scavenging activity was calculated using the following equation:

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

where, A_0 is the absorbance of the control, and A_1 is the absorbance of the test sample. The concentration required to inhibit 50% of DPPH radicals (half-maximal inhibitory concentration [IC_{50}]) was determined by non-linear regression analysis using GraphPad Prism 9 software.

Ferric reducing antioxidant power (FRAP) assay

The FRAP of the samples was determined based on their ability to reduce ferric ions to ferrous ions. A dilution series of the test samples was prepared. To each test tube, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) was added, followed by 2.5 mL of 1% potassium ferricyanide solution. The reaction mixtures were vortexed and incubated at 50°C for 20 min. After incubation, 2.5 mL of 10% trichloroacetic acid was added to each tube to terminate the reaction.

The mixtures were centrifuged at 3,000 rpm for 10 min. Subsequently, 2.5 mL of the supernatant was transferred into fresh test tubes, and 0.5 mL of FeCl_3 solution was added. The formation of a bluish-green color indicated ferric ion reduction. Absorbance was measured at 700 nm using a spectrophotometer. Ascorbic acid was used as the positive control, and distilled water served as the negative control. All experiments were performed in triplicate. The antioxidant activity was expressed as a normalized percentage, and IC_{50} values were calculated using nonlinear regression analysis in GraphPad Prism 9 software.

Anti-inflammatory activity

Inhibition of protein denaturation assay

The *in vitro* anti-inflammatory activity of the samples was assessed by evaluating their ability to inhibit protein denaturation using the egg albumin denaturation method. The reaction mixture consisted of 0.5 mL of ethyl acetate extract of *M. oleifera*-mediated AgNPs (*M. oleifera* leaf ethyl acetate extract [MOET]-AgNPs; 1 mg/mL) and 0.45 mL of a 5% aqueous solution of BSA. The pH of the mixture was adjusted to 6.3 using 0.1 N hydrochloric acid. The mixtures were incubated at 37°C for 20 min, followed by heating at 57°C for 30 min to induce protein denaturation. After cooling to room temperature, the reaction mixtures were transferred to 96-well microplates, and absorbance was measured at 660 nm using a microplate reader. Diclofenac sodium (1000 $\mu\text{g/mL}$) was used as the standard reference drug, while the control consisted of a reaction mixture without the test sample, containing distilled water instead. The percentage inhibition of albumin denaturation was calculated using the following formula:

$$\% \text{ Inhibition} = (A_c - A_t / A_c) \times 100$$

Where, A_c = Absorbance of the control; A_t = Absorbance of the test sample

RESULTS

UV-visible spectrophotometer analysis

UV spectroscopy was initially used to see the NPs, and it proved to be a very useful tool for studying NPs. As the leaf extracts continued to vary with the fluid arrangement of the silver molecule complex, *M. oleifera*'s color changed from red to brown. The creation of AgNPs as a result of the surface plasma ambiances' agitation is seen in purple [Figure 2]. The UV-visible spectrum of AgNPs as a stretch component was recorded using a quartz glass cuvette filled with pure water. At 90°C, the reaction between a 5 mL leaf removal and a 95 mL AgNO_3 suspension was seen. *Tenuiflorum* L. O. At 421 nm, the UV-visible range retention is measured.

FT-IR spectrophotometer analysis

With only slight variations in absorbed wavelengths and % transmittance, the bands created by various sorts are almost identical. The FTIR range of *M. oleifera*. Purple AgNPs are displayed in Figure 3. The O-H extension, which includes H-fortified alcohols and phenols, is defined as the wavelength range at 3263 cm^{-1} . The band 2928.23 cm^{-1} is designated for the carboxylic acid O-H stretching. The N-H twisting of vital amines is shown by the wavelength range at 1636.45 cm^{-1} . The C extending of alcohols contains carboxylic acids in the range of $1236.78\text{--}1074.41\text{ cm}^{-1}$.

TEM analysis

High-thickness AgNPs combined with the leaf extract were visible in the TEM images, suggesting the establishment of a



Figure 1: *Moringa oleifera* leaf and commercially available brand

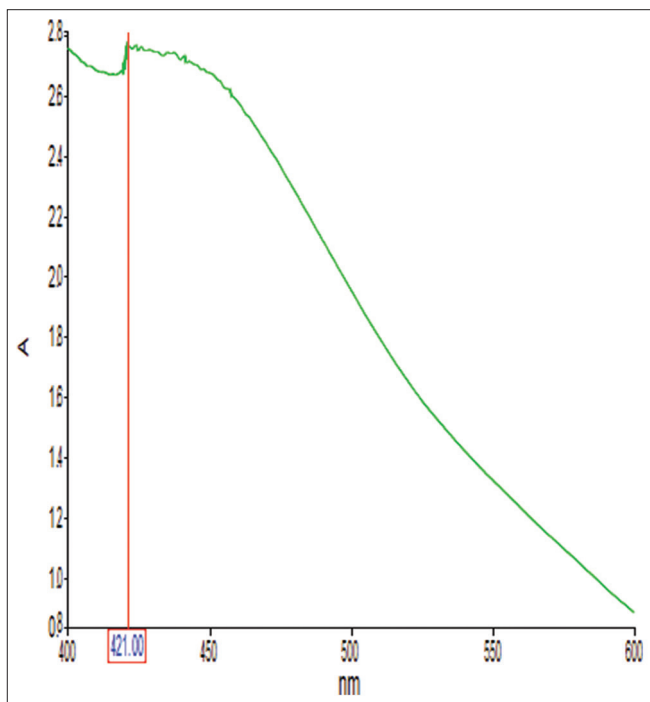


Figure 2: Ultraviolet spectrum absorption of silver nanoparticles obtained from leaves of *Moringa oleifera*

positive silver nanostructure. The TEM image of *M. oleifera* leaves extract synthesised NPs clearly shows the development of the leaky surface with round NPs and partially textured round NPs. They varied from 20.46 to 80.42 nm with ease [Figure 4].

EDX spectroscopy

The entire chemical structure of embedded AgNPs and the material's sanctity are both reflected in the EDX spectra. 93.5% of the AgNP samples made from leaves of *M. oleifera* [Figure 5 and Table 1] are present in the amalgamation at the time of analysis, according to the EDX study. It demonstrated the value of the contained sample by revealing a considerable amount of silver.

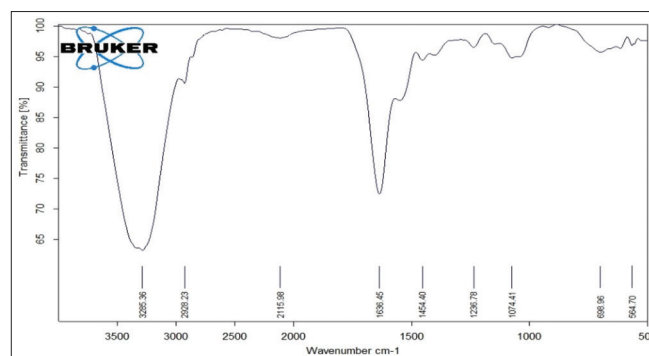


Figure 3: Fourier transform infrared spectrum of silver nanoparticles obtained from leaves of *Moringa oleifera*

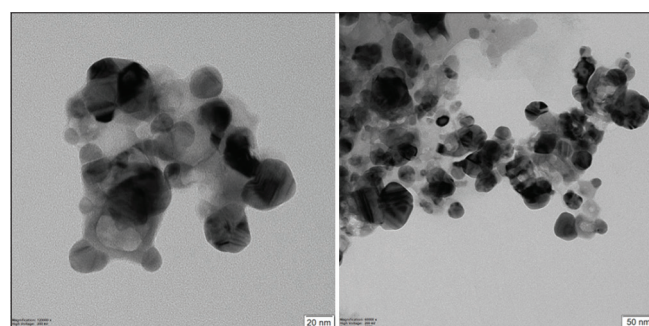


Figure 4: Transmission electron microscopy image of silver nanoparticles obtained from leaves of *Moringa oleifera*

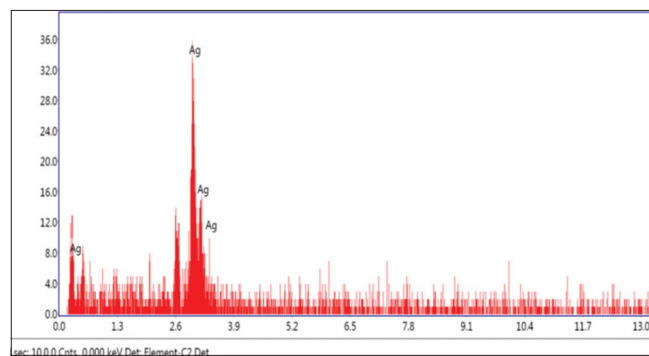


Figure 5: Energy dispersive X-ray spectra of silver nanoparticles obtained from leaves of *Moringa oleifera*

Particle size and zeta potential analysis

According to the findings, the PDI is 0.263, and the average particle diameter is 102 nm. The average particle size and PDI indicated that the produced AgNPs were monodispersed in their native state [Figure 6]. The surface charge of the biogenic AgNPs was measured using zeta potential analysis. Accordingly, it was discovered that the produced AgNPs zeta potential was -22.7 mV [Figure 7]. Furthermore, the capping effect of the biomolecules in the leaf extract of *M. oleifera* is responsible for the negative charge of the biogenic NPs.^[19] The AgNPs' measured negative surface charge demonstrated their electrostatic repulsion.^[20]

XRD analysis

XRD analysis was used to examine the freshly synthesized AgNPs' phase distribution, crystallinity, and purity. Figure 8 shows the AgNPs derived from *M. oleifera*'s XRD patterns. The characteristic XRD pattern of pure NPs was used to determine that the NPs had a cubical structure, were crystalline in nature, and contained no such impurities. The peaks were observed at 2θ values of 38.32° (Ag_2O), 44.67° (Ag/AgO), 64° (Ag_2O), 74.21° (Ag), 77.14 , and 78.62° (Ag)

Table 1: Energy dispersive X-ray result of AgNp obtained from leaves of *Moringa oleifera*

Metal	Element weight %	Atomic %	Error %
Ag	100.00	100.00	12.01

AgNp: Silver nanoparticles

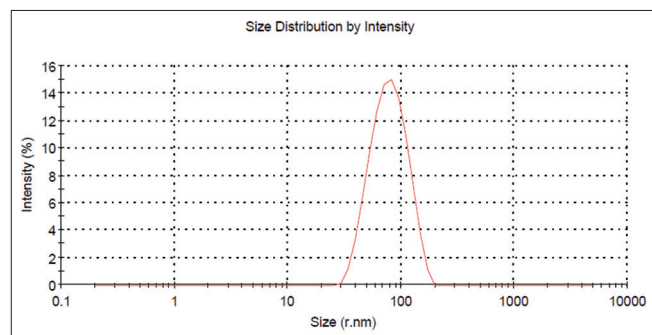


Figure 6: Particle size of silver nanoparticles obtained from leaves of *Moringa oleifera*

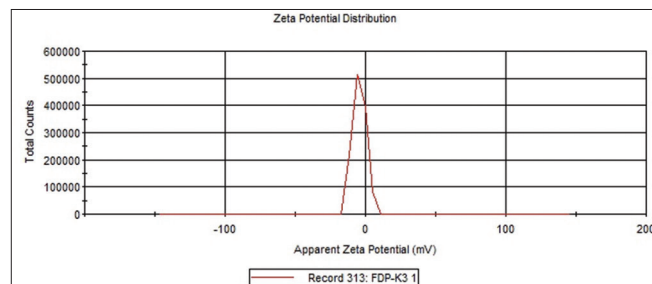


Figure 7: Zeta potential of silver nanoparticles obtained from leaves of *Moringa oleifera*

correspond to Ag (111), (200), (220), (311), and (222). High intensity at 38.45° reflection indicates that the crystallites are mainly oriented in this plane. Dorobantu *et al.* reported that the formation of Ag from AgO is mainly due to lower temperatures below 30°C .^[21] The reflections are broader, revealing the smaller size of AgNP crystals.

Anti-oxidant and anti-inflammatory activity

The purified and characterized AgNPs were subsequently evaluated for the *in-vitro* antioxidant and anti-inflammatory activities of *M. oleifera* leaves extract, AgNPs synthesized using *M. oleifera* leaves, and standard reference compounds. The values are depicted as follows in Tables 2-4, respectively.

DISCUSSION

M. oleifera has been extensively utilized in both traditional and modern applications due to its notable antimicrobial properties, which are largely attributed to its phytoconstituents. However, studies focusing on the synthesis of AgNPs (AgNPs using *M. oleifera*) remain limited. In the present investigation, AgNPs were successfully synthesized using the leaf extract of *M. oleifera*. AgNPs have gained considerable attention for a wide range of applications, including cancer diagnosis, cancer therapy, and drug delivery systems. The biosynthesized AgNPs were initially characterized using UV-visible spectroscopy, FTIR spectroscopy, TEM, EDX analysis, and particle size analysis to confirm NP formation from *M. oleifera* leaf extract. The synthesized AgNPs exhibited characteristic features such as nanoscale particle size, elemental silver composition with high purity, and predominantly spherical to irregular spherical morphology.^[22]

The purified and characterized AgNPs were subsequently evaluated for the *in-vitro* antioxidant and anti-inflammatory activities of *M. oleifera* leaves extract, AgNPs synthesized using *M. oleifera* leaves, and standard reference compounds. The findings clearly demonstrate variation in bioactivity depending on the test system and formulation.

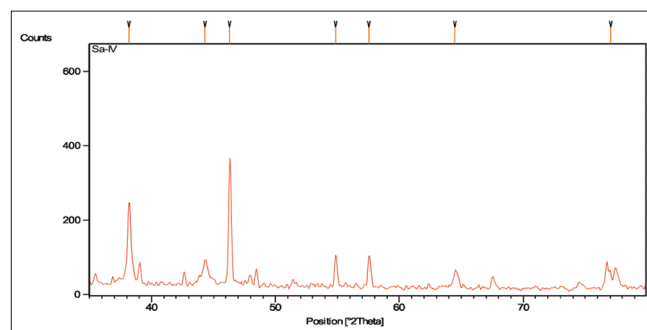


Figure 8: X-ray diffraction pattern of nanoparticles obtained from leaves of *Moringa oleifera*

Table 2: *Moringa oleifera* leaves extract, AgNPs obtained from leaves of *M. oleifera* and Std Vit. C *in vitro* antioxidant activity by DPPH

Sample	Concentration (mg)	Activity (%)*	IC ₅₀ (mg/mL)
MOET	100	47±0.75	169.72
	200	63±0.93	
	300	65±0.82	
MOET AgNPs	100	84±1.24	141.4214
	200	74±1.62	
	300	77±1.67	
Std. Vit-C	50	91±0.64	70.7107
	100	88±0.85	
	150	92±0.74	

*n=3 (Number of times repeated the test procedure).
MOET: *Moringa oleifera* leaves ethyl acetate extract,
AgNPs: Silver nanoparticles, DPPH: 1,1-diphenyl-2-picrylhydrazyl,
IC₅₀: Half-maximal inhibitory concentration

Table 3: *Moringa oleifera* leaves extract, AgNPs obtained from leaves of *M. oleifera* and Std Vit C *in vitro* antioxidant activity by FRAP

Sample	Concentration (mg)	Activity (%)*	IC ₅₀ (mg/mL)
MOET	50	9.2±1.42	120.4229
	100	18±1.65	
	150	-27±2.85	
	200	12±1.87	
MOET AgNPs	50	-143±2.65	205.7849
	100	-12.1±2.21	
	150	-155.01±2.48	
	200	46±2.45	
Std Vit- C	50	33±1.34	97.73
	100	25±1.45	
	150	19±1.52	

*n=3 (Number of times repeated the test procedure).
MOET: *Moringa oleifera* leaves ethyl acetate extract,
AgNPs: Silver nanoparticles, FRAP: Ferric reducing antioxidant power, IC₅₀: Half-maximal inhibitory concentration

DPPH assay

In the DPPH radical scavenging assay, both MOET and Biogenic AgNPs from MOET-AgNPs exhibited concentration-dependent antioxidant activity, indicating their ability to donate hydrogen atoms or electrons to neutralize free radicals. However, MOET-AgNPs showed markedly higher scavenging activity than the crude extract at all tested concentrations. At 100 mg, MOET-AgNPs demonstrated 84 ± 1.24% inhibition compared to 47 ± 0.75% for MOET, reflecting nearly a two-fold enhancement. The improved activity of MOET-AgNPs was further supported by a lower IC₅₀ value (141.42 mg/mL) compared to MOET (169.72 mg/mL), suggesting greater antioxidant potency.

Table 4: *Moringa oleifera* leaves extract, AgNPs obtained from leaves of *M. oleifera*, and Std diclofenac *in vitro* anti-inflammatory activity

Sample	Concentration (mg)	Activity (%)*	IC ₅₀ (mg/mL)
MOET	100	43±1.25	192.03
	200	53±1.68	
	300	59±1.32	
MOET AgNPs	100	45±2.44	316.028
	200	45±1.64	
	300	50±1.872	
Std diclofenac sodium	50	84±0.34	106.67
	100	82±1.15	
	150	78±2.74	

*n=3 (Number of times repeated the test procedure).
MOET: *Moringa oleifera* leaves ethyl acetate extract,
AgNPs: Silver nanoparticles, FRAP: Ferric reducing antioxidant power, IC₅₀: Half-maximal inhibitory concentration

This enhancement may be attributed to the synergistic effect between AgNPs and phytoconstituents, such as flavonoids, phenolics, and ascorbate-like compounds, which act as capping and stabilizing agents during NPs synthesis. The increased surface area and reactivity of AgNPs likely facilitate better interaction with DPPH radicals. As expected, standard vitamin C exhibited the highest antioxidant activity, with an IC₅₀ of 70.71 mg/mL, confirming the validity of the assay. Nevertheless, the strong activity of MOET-AgNPs highlights the advantage of green-synthesized NPs over crude plant extracts.

FRAP assay

In contrast to the DPPH results, the FRAP assay revealed variable and inconsistent reducing power for both MOET and MOET-AgNPs. MOET showed relatively low FRAP values, with some concentrations exhibiting negative or reduced activity, indicating limited ferric ion-reducing capacity. Similarly, MOET-AgNPs displayed fluctuating responses, with pronounced negative values at certain concentrations, though a notable increase (46 ± 2.45%) was observed at 200 mg.

These variations may be due to assay-specific mechanisms, as FRAP primarily measures electron-donating capacity under acidic conditions, which may not favor all antioxidant constituents present in the extract or NPs formulation. In addition, the presence of silver ions or NPs aggregation at certain concentrations may interfere with the redox reaction, leading to anomalous readings.

Vitamin C, however, showed consistent and significantly higher FRAP activity across all concentrations, reinforcing its strong reducing potential. Overall, the FRAP results suggest that while MOET and MOET-AgNPs possess antioxidant

potential, their reducing power is comparatively weaker and more assay-dependent than their radical scavenging ability.

Anti-inflammatory activity

The *in vitro* anti-inflammatory evaluation revealed that MOET exhibited moderate, concentration-dependent anti-inflammatory activity, with inhibition increasing from $43 \pm 1.25\%$ at 100 mg to $59 \pm 1.32\%$ at 300 mg. The IC_{50} value of 192.03 mg/mL indicates significant efficacy, likely due to the presence of bioactive phytochemicals such as phenolic acids, flavonoids, and isothiocyanates known to modulate inflammatory mediators. MOET-AgNPs, however, demonstrated comparatively lower anti-inflammatory activity, with marginal increases across concentrations and a higher IC_{50} value (316.03 mg/mL). This suggests that NPs, while beneficial for antioxidant activity, may not proportionally enhance anti-inflammatory effects. Possible explanations include altered bioavailability of active compounds, steric hindrance by NPs capping agents, or reduced interaction with inflammatory proteins in the assay system. Standard Diclofenac sodium showed significantly higher inhibition with a much lower IC_{50} (106.67 mg/mL), serving as a positive control and highlighting the comparatively mild anti-inflammatory nature of the plant-based samples.

CONCLUSION

Collectively, this study results indicate that green-synthesized MOET-AgNPs significantly enhance antioxidant activity, particularly in free-radical scavenging assays, compared to the crude extract. However, anti-inflammatory activity was more pronounced in the crude extract than in the NPs formulation. These findings suggest that NPs synthesis selectively increases certain biological properties while potentially weakened others. The study underscores the importance of assay-specific evaluation and supports the potential of *M. oleifera*-mediated AgNPs as antioxidant agents, while the crude extract may remain more suitable for anti-inflammatory applications. Further mechanistic and *in-vivo* studies are guaranteed to elucidate the molecular basis of these observations.

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