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Unveiling mystical medicinal plant *Moringa oleifera Lam.* (Moringaceae) leaf bioactive compound beneficial in alleviating gastrointestinal diseases from Satpura Hills

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Abstract

In this study, crude extracts from the leaves of Moringa oleifera Lam in different solvents such as water, ethanol, acetone, benzene, and n-hexane were obtained using the Soxhlet extraction method. Various macro and microelements like Ca-4.13 mg/L, Fe-8.34 mg/L, K-6.41 mg/L, Mg-3.99 mg/L, Na-4.21 mg/L, Zn-0.76 mg/L, and Cu-0.46 mg/L were estimated by using atomic absorption spectroscopy. The ethanolic leaf extracts were screened using different preliminary tests for analysis. From Phytochemical and FT-IR analyses confirmed the presence of bioactive compounds. The total phenol content was analyzed by colorimetric total phenolic assay performed using the Folin–Ciocalteu (F–C) reagent, and the total flavonoid content of the extract was determined by the aluminum chloride calorimetric method. Vitamin E, Tetracosane, Taraxasterol Acetate, Linolenic acid, Phytol, 1-Pentacosene, 1-Heptacosene, 1-Nonacosene were the major compounds obtained from the leaf ethanolic extract using GC-MS. The DPPH (2,2-diphenyl- 1-picrylhydrazyl), hydrogen peroxide (H_2O_2), nitric oxide (NO), and ferric reducing antioxidant power (FRAP) assays showed lower DPPH and ferric-reducing antioxidant activity than the standard pure butylated hydroxytoluene. Antimicrobial screening was performed against fungi; bacillus subtilis, staphylococcus aureus, pseudomonas aeruginosa and Escherichia coli showed zones of inhibition at 16mm, 14mm, 15,mm and 15 mm, respectively.

Keywords: *Moringa oleifera Lam., Leaf extract, Antimicrobial, Antioxidant, FTIR, GC-MS, Atomic absorption spectroscopy.*

Introduction

Livestock rearing and herding are a crucial integral part of rural India, and low socioeconomic tribes are fully dependent on their livestock for their bread and butter. These tribes face a huge financial problem when it comes to treating their animals with modern medicines available in the market, as they are expensive and not pocket-friendly. Livestocks are exposed to many diseases, including viruses, bacteria, parasites, and protozoa. Few such ailments which are a huge headache to these livestock owners are Foot and mouth diseases, ruminants, hemorrhagic septicemia [HS], intussusception, winter dysentery, parvovirus, bloating, flatulence etc. The tribes from Gonapur of tehsil Prabhat pattan which lies at the frontiers of Maharashtra and Madhya Pradesh but has ethnicity of Maharashtra use ethnoveterinary medicines to treat their livestock, many herbal plants are available in this region one of these which fascinated us was *Moringa oleifera Lam.* commonly known as "drumstick tree," an important medicinal plant belongs to the family Moringaceae under the order Brassicales, is widely distributed and cultivated throughout the tropical and subtropical region of South Asia, especially in Indian subcontinent. The plant is known as Haritashaaka, Raktaka, and Akshiva in Ayurveda and Sahajan in the Unani system of medicine [1]. Normally, the tree is small to medium in size, the leaves are naturally trifoliate, and the flowers are born on an inflorescence 10–25 cm long [2]. The powdered leaves are used to make many beverages, of which "Zija" is the most popular in India [3]. Recent pharmacological studies have revealed that different extracts of *Moringa oleifera Lam.* exhibit different pharmacological activities, such as antimicrobial [4], antifungal [5], and antioxidant [6] activities, and our aim was to unveil the hidden bioactive compounds found in the leaf of *Moringa oleifera Lam.* using different quantitative and qualitative techniques, such as atomic absorption spectroscopy, FTIR, GC-MS and Antimicrobial and antioxidant tests, respectively. This might be helpful in identifying compounds that are significant in curing various gastrointestinal diseases in livestock from the tribes of Gonapur.

Materials and Methods

Sample collection and extraction

Leaves of *Moringa oleifera Lam.* were collected from Gonapur (Satpura hills) and verified using flora [7]. Used by local ethnoveterinary practitioners for gastrointestinal diseases. After washing with hydro-alcohol, the leaves were dried in the shade. A 25 g sample was extracted using a Soxhlet apparatus with ethanol solvent, and the extracts were concentrated at room temperature [8], [9].

Chemicals and reagents

All the chemicals and reagents were of analytical grade and obtained commercially from Molychem Badlapur, Dist. Thane, Maharashtra, India.

Phytochemical analysis

The ethanolic extract of the leaves was subjected to the detection of various phytoconstituents, such as alkaloids, cardiac glycosides, coumarins, flavonoids, phenolic compounds, reducing sugars, saponins, steroids, tannins, and terpenoids, and proteins etc. [10-14]

Atomic absorption spectroscopy (AAS)

The leaf samples of Moringa oleifera were analyzed for Ca, Fe, K, Mg, Zn, Na, and Cu using the digestion method. Two grams of each sample were treated with 15 ml of 65% nitric acid and 5 ml of 30% hydrogen peroxide and then heated at 250 °C for 3 hours until clear. The digests were filtered (Whatman No. 42), diluted with distilled water, and analyzed using a Perkin Elmer AA300 atomic absorption spectrophotometer. [15], [16]

FT-IR analysis for phytochemical screening

FTIR (Fourier Transform Infrared Spectroscopy (FTIR) analysis of the ethanolic extract was performed using Bruker ATR mode FTIR to confirm the class of phytochemicals. The dried extract was ground into a fine powder using an agate mortar along with a standard KBr tablet (1:8) and examined with a scan time (10 min) at a resolution of 4 cm-1 ranging from 4000 to 400 cm−1.27 the room was kept at a controlled ambient tem¬perature (25°C) and relative humidity (30%) [17].

Total Phenolic Content (TPC) Estimation:

TPC was measured using the modified Folin–Ciocalteu method. A 2 mL plant extract (10 mg in 20 mL ethanol) was mixed with 2 mL of 10% Folin–Ciocalteu reagent. After 1 minute, 4 mL of 7.5% sodium carbonate was added and the volume was adjusted to 10 mL with distilled water. The mixture was incubated at 40°C for 30 min, and the absorbance was measured at 750 nm (Shimadzu UV-Vis 1800). Gallic acid was used to prepare the calibration curve (50–500 µg/mL). The results are expressed as mg gallic acid equivalents (GAE)/g dry extract. TPC was calculated using the formulas: $C_1 = (y - c) / m$ and $C = (C_1 \times V) / m$ [18-20].

Total Flavonoid Content (TFC) Estimation:

This was determined using the aluminum chloride ($AlCl_3$) colorimetric method, which forms a yellow complex with flavonoids, measured at 510 nm. The assay involved mixing 2 mL of the sample extract (10 mg in 20 mL ethanol) with $NaNO_2$, $AlCl_3$, NaOH, and distilled water to a final volume of 10 mL. After 20 minutes of incubation at room temperature, absorbance was recorded using a UV-Vis spectrophotometer. A quercetin standard curve (50–500 µg/mL) was used for calibration. TFC was expressed as mg quercetin equivalents (mg QE/g dry extract) using the equations: $C_1 = (y - c) / m$ and $C = (C_1 \times V) / m$ [21], [22].

GC-MS analysis

GC-MS analysis was conducted at CSIR-IICT using an Agilent 5977A spectrometer to identify the volatile bioactive compounds in the extracts. The samples were dissolved in ethanol, and 2 µL was injected at an 70 eV ionization energy of 70 eV. Helium (99.999%) was used as the carrier gas at 1.00 mL/min. The injector, ion source, and MS transfer line temperatures were 230 °C, 300 °C, and 280 °C, respectively. The oven was programmed to run from 40 °C to 235 °C in steps, with a total run time of 32 minutes. The compounds were identified by retention time and NIST library comparisons [17].

Antioxidant assays

DPPH Radical Scavenging Assay: Antioxidant activity of the plant extracts was evaluated using a modified DPPH assay [23]. Various extract concentrations (25–125 µg/mL) were mixed with 3 mL of 0.004% DPPH in ethanol. The control contained ethanol instead of the extract. The samples were incubated in the dark for 30 minutes at room temperature, and the absorbance was read at 517 nm. % Inhibition was calculated as: % Inhibition = $[(A_0 - A_1) / A_0] \times 100$, where A_0 = control absorbance, A_1 = sample absorbance. BHT and gallic acid were used as standards. Tests were performed in triplicate, and the results are reported as the mean ± SD.

Nitric oxide (NO) scavenging Assay: Antioxidant activity of plant extracts was evaluated using sodium nitroprusside in phosphate-buffered saline (pH 7.4), which generates NO that reacts with oxygen to form nitrite, detected via the Griess reagent. A phytochemical extract (25–125 µg/mL in ethanol) was incubated with the reaction mixture for 150 minutes at room temperature. After adding Griess reagent (0.5 mL) Griess reagent, the absorbance was measured at 546 nm using a UV-Vis spectrophotometer. Butylated hydroxytoluene and gallic acid were used as the standards. % Inhibition = $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the control absorbance and A_1 is the sample absorbance. Tests were performed in triplicate, and the results are presented as the mean ± SD [23].

Hydrogen Peroxide Scavenging Assay: The Antioxidant activity was assessed by measuring the ability of extracts (25–125 µg/mL) to scavenge H_2O_2 (43 mM), prepared by diluting 2.19 mL of 6% H_2O_2 in 100 mL phosphate buffer (pH 7.4, 1 M). Each reaction contained 1 mL extract, 0.6 mL H_2O_2, and buffer to make up 5 mL. After 15 minutes of incubation, the absorbance was measured at 230 nm. Controls included buffer without H_2O_2 and ethanol blanks. Standard antioxidants (butylated hydroxytoluene (BHT) and gallic acid) were also tested similarly. % inhibition = $[(A_0 - A_1) / A_0] \times 100$. All tests were performed in triplicate, and the results are presented as the mean ± SD [24].
The Ferric Reducing Antioxidant Power (FRAP) assay: The antioxidant potential of the phytochemical extract was evaluated using a modified method by Kumaran and Karunakaran [23], which measures the reduction of Fe^{3+} to Fe^{2+}. Extracts (25–125 µg/mL in ethanol) were mixed with 1% $K_3Fe(CN)_6$ and 0.2 M phosphate buffer (pH 6.6), then incubated at 50 °C for 20 minutes. After addition of 10% TCA, the samples were centrifuged at 3000 rpm for 10 minutes. The supernatant was diluted with water, followed by addition of 0.1% $FeCl_3$. Absorbance was recorded at 700 nm was recorded; higher absorbance indicated greater antioxidant activity via electron-donating ability.

Antimicrobial screening
Antibacterial Activity: The antibacterial potential of the plant extracts (100 mg/mL) was tested using the well diffusion method of Patra et al. [25] against selected bacterial strains. Nutrient agar plates were inoculated with bacterial cultures grown overnight. Wells (5–6 mm) were made using a sterile cork-borer and sealed with 10–20 µL molten agar. Each well received 200 µL of the extract; solvent-only samples served as negative controls. The plates were then incubated at 37°C for 23–30 hours. Zones of inhibition (mm) were measured. The test organisms included *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli*.
Antifungal Assay: A modified disc diffusion method was used to evaluate the antifungal activity of plant extracts against *Aspergillus niger* and *Fusarium pallidoroseum*. The fungi were cultured on PDA and SDA media and incubated at 25–30°C for 3–5 days. A 0.5 turbidity fungal suspension was spread onto sterile agar plates. Sterilized 5 mm filter paper discs loaded with 100 µL of plant extract (100 mg/mL) were placed on the agar. Solvent-only discs served as negative controls. The plates were incubated at 25–30°C for 24–72 hours. Inhibition zones were measured in millimeters, with larger zones indicating stronger antifungal activity. All tests were performed in triplicate for accuracy [26-29].

Result and discussion
In this study, we investigated various phytochemical properties of the leaves of *Moringa oleifera Lam.* The results of the different parameters are as follows.

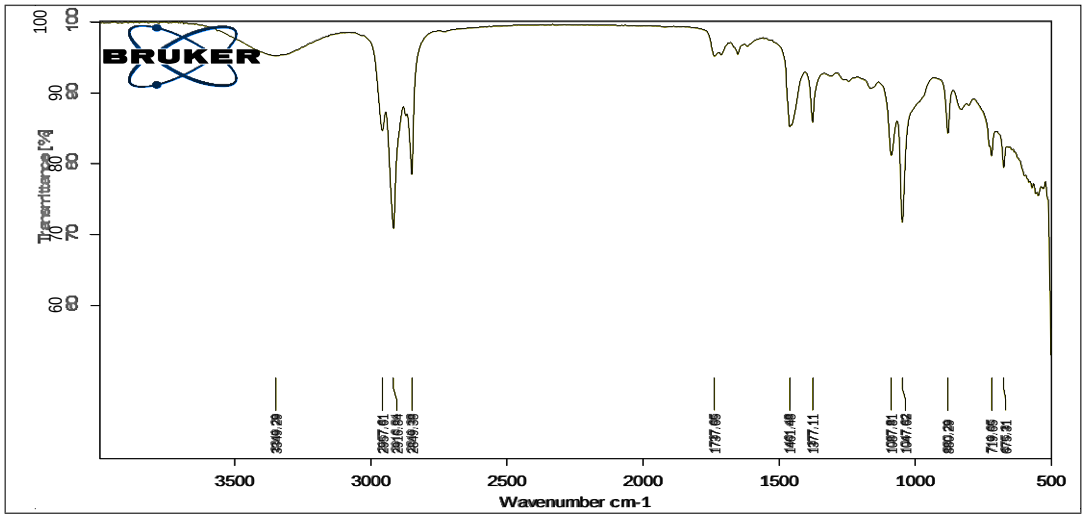
Phytochemical screening
The Preliminary phytochemical constituents revealed the presence of Alkaloids, Glycosides, Phenols, Saponins, Tannins, Flavonoids, Triterpenoids, Proteins, Carbohydrates. The total phenolics (91.24±0.95 mg/g gallic acid equivalents) and flavonoids (9.63±1.15 mg/g Quercetin equivalents).

Element analysis by Atomic Absorption Spectroscopy-

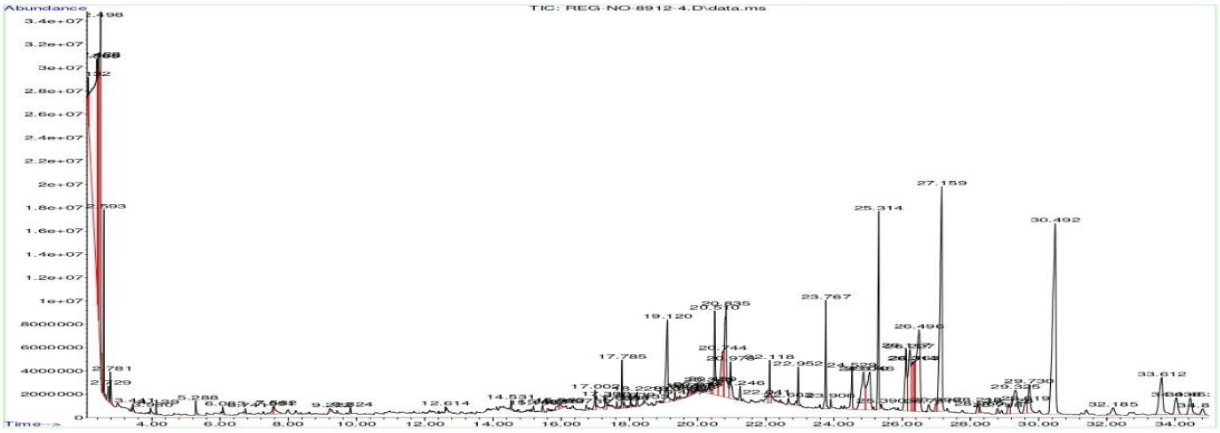
Elements analyzed	Quantity of the element (mg/L)
Calcium (Ca)	4.13 mg/L
Iron (Fe)	8.34 mg/L
Potassium (K)	6.41 mg/L
Magnesium (Mg)	3.99 mg/L
zinc (Zn)	0.76 mg/L
Sodium (Na)	4.21 mg/L
Copper (Cu)	0.46 mg/L

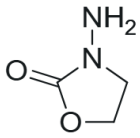
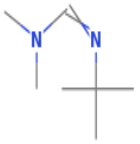
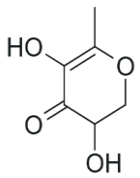
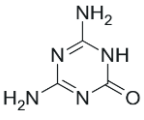
FTIR spectra of ethanolic extract of Moringa oleifera Lam. Leaf

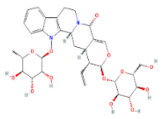
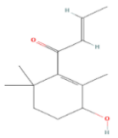
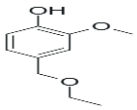
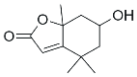
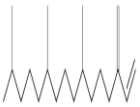
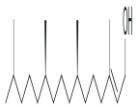
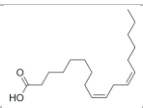
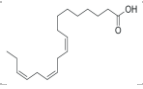
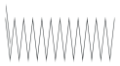
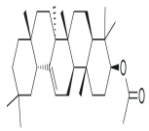
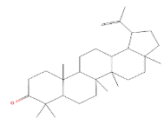
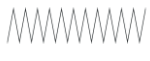
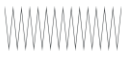
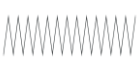
Sr. No.	Wave number (cm-1)	Group	Compound
	3349.29	N-H	Secondary amine
2	2957.61	C-H	Alkene
3	2910.84	C-H	Alkene
4	2849.38	C-H	Alkene
5	1737.65	C=O	Anhydride
6	1461.48	C-H	Alkene
7	1377.11	O-H	Phenol
8	1087.81	C-O	Secondary alcohol
9	1047.62	CO-O-CO	Anhydride
10	880.29	C-H	1,2,4-trisubstituted
11	719.65	C=C	Alkene
12	675.31	C-Br	Halo compound

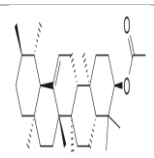
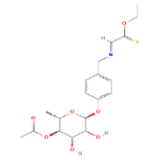
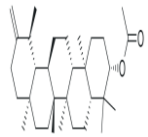
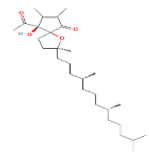
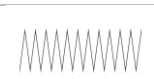
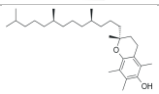
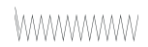
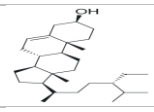
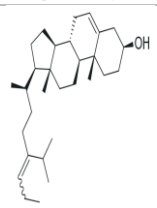
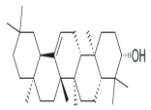


Gas chromatography-mass spectrometry analysis of ethanolic extract of *Moringa oleifera Lam.*



Sr. No.	Retention Time	Peak area (%)	IUPAC name	Category	Molecular Formula	Molecular weight	Probable structure
1	3.961	1.15	3-Amino-2-oxazolidinone	Oxazolidinones	$C_3H_6N_2O_2$	102.13 g/mol	
2	7.551	1.21	N'-tert-Butyl-N,N-dimethylformamidine	Formamidine	$C_7H_{16}N_2$	128.2153	
3	9.221	2.03	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Flavonoid	$C_6H_8O_4$	144.12 g/mol	
4	14.534	1.15	1,3,5-Triazin-2(1H)-one, 4,6-diamino-	Phenol	$C_3H_5N_5O$	127.1047	

5	15.185	1.12	N,α-L-rhamnopyranosyl vincosamide	Alkaloid	$C_{12}H_{14}O_4$	222.24 g/mol	
6	15.454	2.15	3-Hydroxy-.beta.-damascone	Phenol	$C_{13}H_{20}O_2$	208.30 g/mol	
7	15.961	1.19	Phenol, 4-(ethoxymethyl)-2-methoxy-	Phenol	$C_{10}H_{14}O_3$	182.2164 g/mol	
8	17.309	1.26	(-)-Loliolide	Phenol	$C_{11}H_{16}O_3$	196.24 g/mol	
9	17.782	1.66	Neophytadiene	Terpene	$C_{20}H_{38}$	278.5 g/mol	
10	19.117	2.40	n-Hexadecanoic acid	Lipid	$C_{16}H_{32}O_2$	256.42 g/mol	
11	19.709	1.05	Alpha-Amyrone	Terpene	$C_{30}H_{48}O$	424.7 g/mol	
12	20.511	2.00	Phytol	Terpene	$C_{20}H_{40}O$	296.5 g/mol	
13	20.741	2.16	9,12-Octadecadienoic acid (Z,Z)-	Lipid	$C_{18}H_{32}O_2$	280.4455	
14	20.833	3.27	Linolenic acid	Lipid	$C_{18}H_{30}O_2$	278.43 g/mol	
15	23.766	1.71	n - pentacosanene	*Alkane*	$C_{25}H_{52}$	352.7 g/mol	
16	24.870	2.26	Olean-12-en-3-ol, acetate, (3.beta.)- / beta-Amyrin acetate	Terpene	$C_{32}H_{52}O_2$	468.7 g/mol	
17	25.048	2.31	Lup-20(30)-en-3-one	Terpene	$C_{30}H_{48}O$	424.7 g/mol	
18	25.311	3.66	Tetracosane	Lipid	$C_{24}H_{50}$	338.7 g/mol	
19	26.120	1.94	Nonacosane	Lipid	$C_{29}H_{60}$	408.8 g/mol	
20	26.120	1.94	n-Octacosane	Lipid	$C_{28}H_{58}$	394.8 g/mol	

21	26.225s	2.82	Urs-12-en-3-ol, acetate, (3beta)-	Terpene	$C_{32}H_{52}O_2$	468.8 g/mol	
22	26.317	1.67	niaziminin A	Glycoside	$C_{19}H_{25}NO_7S$	411.5 g/mol	
23	26.494	5.23	Taraxasterol Acetate	Terpene	$C_{32}H_{52}O_2$	468.8 g/mol	
24	26.784	1.27	Alpha-Tocospiro A	Phenol	$C_{29}H_{50}O_4$	462.7 g/mol	
25	27.158	9.62	Tetracosane	Lipid	$C_{24}H_{50}$	338.7 g/mol	
26	29.729	1.17	Cyclotetracosane	Lipid	$C_{24}H_{48}$	336.6 g/mol	
27	30.492	9.87	Vitamin E	Phenol	$C_{29}H_{50}O_2$	430.71 g/mol	
28	33.609	2.23	1-Nonacosene	Lipid	$C_{29}H_{58}$	406.8 g/mol	
29	34.036	1.85	gamma-Sitosterol	Steroid	$C_{29}H_{50}O$	414.7 g/mol	
30	34.450	1.89	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	Sterol	$C_{30}H_{50}O$	426.7 g/mol	
31	34.812	1.30	beta-Amyrin	Terpene	$C_{30}H_{50}O$	426.7 g/mol	

Antioxidant activity

The antioxidant potential of the plant extracts was calculated using the rough DPPH, NO, H_2O_2 and FRAP assays. The DPPH assay was used to measure the radical scavenging properties of the extract, and the FRAP assay was used to assessed the reducing ability of the extracts. In the present study, the ethanol extract showed antioxidant activity, which was but less than that of the standard antioxidants.

DPPH assay of ethanolic extract of Moringa oleifera Lam. Leaf

Sample	Concentration (mg/ml)	% inhibition	IC50(mg/ml)
Ethanol extract	25	41.75	59.01±0.55
	50	46.23	
	75	55.19	
	100	61.88	
	125	69.78	
Gallic acid			31.17±0.25
Butylated hydroxytoluene (BHT)			24.53±0.28

Nitric oxide assay of ethanolic extract of Moringa oleifera Lam. Leaf

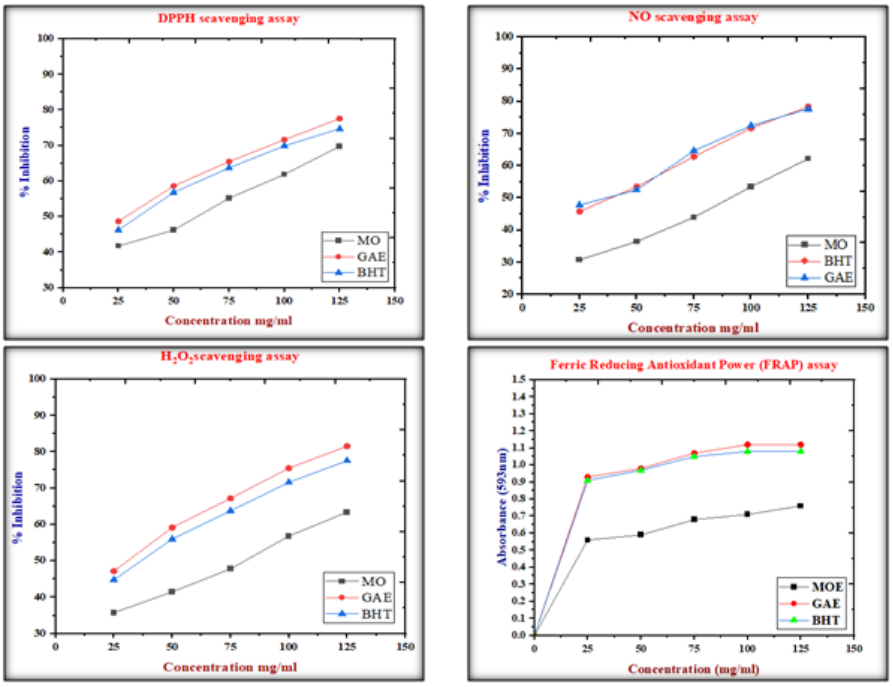
Sample	Concentration (mg/ml)	% inhibition	IC50(mg/ml)
Ethanol extract	25	30.74	89.62±0.42
	50	36.47	
	75	43.96	
	100	53.49	
	125	62.14	
Gallic acid			38.12±0.38
Butylated hydroxytoluene (BHT)			35.22±0.60

Hydrogen peroxide assay of ethanolic extract of Moringa oleifera Lam. Leaf

Sample	Concentration (mg/ml)	% inhibition	IC50(mg/ml)
Ethanol extract	25	35.74	79.01±0.65
	50	41.48	
	75	47.78	
	100	56.82	
	125	63.39	
Gallic acid			36.31±0.15
Butylated hydroxytoluene (BHT)			27.73±0.10

FRAP assay of ethanolic extract of Moringa oleifera Lam. Leaf

Sr. No.	Concentration in mg/ml	Blank	Absorbance of plant extract	Absorbance of Butylated hydroxytoluene	Absorbance of Gallic acid
1	25	0.32	0.56	0.91	0.93
2	50		0.59	0.97	0.98
3	75		0.68	1.05	1.07
4	100		0.71	1.08	1.12
5	125		0.76	1.08	1.12

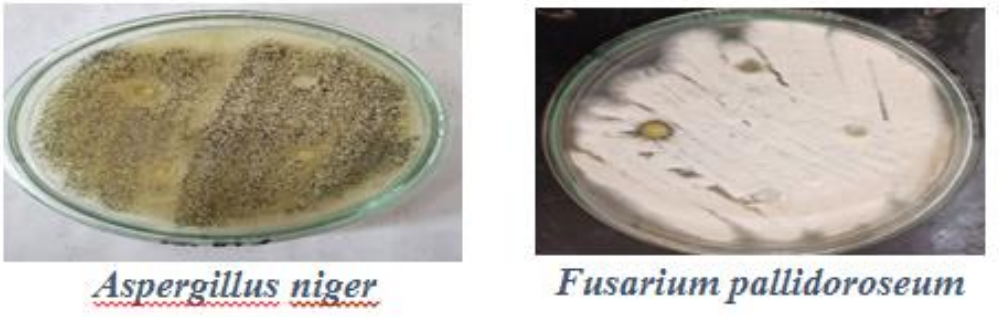


Antimicrobial activity of ethanolic leaf extracts of *Moringa oleifera Lam.* against the selected strains of bacteria-

Sample	Gram positive Bacteria		Gram negetive Bacteria	
Ethanolic extract of leaf	*Bacillus subtilis*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*
Zone of Inhibition	16mm	14mm	15mm	15mm

Antifungal Antimicrobial activity of ethanolic leaf extracts of *Moringa oleifera Lam.* against the selected strains of fungi-

Sample	Fungal strain	
Ethanolic extract of leaf	*Aspergillus niger*	*Fusarium pallidoroseum*
Zone of Inhibition (Mean)	6mm	11mm



Discussion

This study highlights the diverse phytochemical and biological properties of Moringa oleifera Lam. leaf extract. Preliminary screening revealed the presence of key bioactive compounds, such as alkaloids, flavonoids, phenols, saponins, tannins, and triterpenoids, with notable levels of total phenolics (91.24 mg/g GAE) and flavonoids (9.63 mg/g QE), consistent with previous studies [30], [31]. Elemental analysis indicated the presence of essential minerals such as iron, potassium, calcium, and magnesium, supporting the plant's nutritional significance and aligning with the findings of Aslam et al. [32]. FTIR and GC-MS analyses have confirmed a wide array of functional groups and compounds, including phenolics, terpenes, flavonoids, and fatty acids, many of which are associated with antioxidant and antimicrobial activities [33], [34]. The extract exhibited moderate antioxidant activity in DPPH, NO, H_2O_2, and FRAP assays, which supports its ability to scavenge free radicals and reduce oxidative stress, as also reported by Verma et al. [35]. Moreover, the extract demonstrated effective antibacterial activity against both Gram-positive and Gram-negative strains, with comparatively lower antifungal activity, corroborating its traditional use in folk medicine [35], [37]. These results validate the therapeutic potential of Moringa oleifera and reinforce its relevance as a bioactive, rich, and multifunctional medicinal plant.

Conclusion

Moringa oleifera Lam. leaf extract exhibits a rich phytochemical profile, essential minerals, and notable biological activities including antioxidant, antibacterial, and mild antifungal properties. These findings support its traditional medicinal uses and highlight its potential for development in natural health products and therapeutic formulations.

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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