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LC-ESI-MS profiling and antioxidant activity assessment of *Moringa oleifera* leaf powder

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Abstract
Bioactive compounds from medicinal plants find diverse uses in food, cosmetics and pharmaceuticals. *Moringa oleifera*, a Himalayan-native resilient species, provides remarkable nutritional and medicinal value. This study evaluates antioxidant potential using DPPH assay and profiles phytochemicals in leaf powder via LC-ESI-MS. Mature leaves collected from West Bengal, India, were shade-dried, methanol-extracted, and analysed with a XEVO G2-XS QT of system. A total of 67 bioactive compounds, including flavonoids, phenolic acids, alkaloids, and glycosides were identified, with key molecules like Rutin, Naringenin and Malvidin showing high relative intensities (≥ 99%). Notably, 30 previously unreported phytochemicals were detected within the 25–100% relative intensity range. The extract demonstrated strong antioxidant activity, with an IC_{50} value of 0.5295 mg/mL, outperforming earlier studies. The presence of diverse metabolites, including glycosylated and iso-flavonoids, highlights the therapeutic relevance of *M. oleifera* leaf powder. These findings enhance knowledge and support its use in functional foods and therapeutic products.

Keywords: *Moringa oleifera,* Phytochemical, LC-ESI-MS analysis, Mass Bank database, DPPH assay, Radical scavenging activity

Introduction
Moringa, the sole genus of the *Moringaceae* family [1], originates from the Himalayan foothills of North India and has spread widely across tropical and subtropical regions, including Africa, Southeast Asia and South America [2]. Its exceptional adaptability to diverse environmental conditions ranging from arid and humid climates to nutrient deficient soils makes it an important crop for regions facing climate stress [3, 4]. Among its species, *Moringa oleifera* widely known as the drumstick tree, Benzolive or Sajnais particularly valued for its fast growth, drought tolerance and resistance to high temperatures [4]. Due to its wide cultivation and environmental resilience, *M. oleifera* has gained recognition as a cosmopolitan and climate-smart species. Often referred to as the "tree of life", it offers a broad spectrum of nutritional, medicinal, agronomic and industrial benefits [5, 6].

The *Moringa oleifera* leaves are its most utilized part due to their high nutritional content and versatility [7]. They can be cooked in soups, and eaten fresh in salads stews or brewed into infusions for medicinal purposes. Dried *Moringa oleifera* leaves are widely used to enrich foods due to their high antioxidant levels [4, 7], protein content comparable to soybeans and abundance of essential amino acids like methionine, making them a nutritious addition to various dietary applications [5]. Additionally, they are rich in important minerals such as calcium, phosphorus, potassium and iron [7]. Beyond basic nutrition, the leaves contain diverse bioactive compounds, including carotenoids, ascorbic acid, glucosinolates and phenolic acids (e.g., ellagic, gallic, ferulic and chlorogenic acids), as well as flavonoids (e.g., quercetin, rutin, kaempferol and vanillin) [5, 6, 7]. Owing to this comprehensive nutrient and phytochemical composition, *M. oleifera* is widely classified as a "super food", offering essential fatty acids (omega-3 and omega-6), tocopherols and various antioxidants [4]. Its multi-functionality and environmental hardiness make it a promising solution to global challenges in nutrition and food security.

Besides its nutritional properties, *M. oleifera* is an accessible and cost-effective health resource, particularly significant in regions with limited access to conventional medicine [8].

Despite growing awareness of its therapeutic potential, more research is needed to clarify its bioactive components, their bioavailability and definitive health impacts [8, 9]. Traditionally, *M. oleifera* leaves have been used to treat headaches, fevers, hemorrhoids, bronchitis, throat inflammation, and conditions related to vitamin C deficiency [10].

Leaf juice is also believed to help regulate blood sugar and reduce gland swelling. The leaves show potential in managing hypertension and cholesterol, with anticancer, anti-inflammatory, diuretic, hepatoprotective, anti-urolithiatic, and analgesic properties [3, 10].

Technological processes influence final product quality, as some bioactive compounds degrade under such treatments, reducing biological value. Drying is a widely used preservation method because it inhibits enzymatic activity and microbial growth, thereby helping retain product stability and quality [11]. Shade drying has been widely recognized as the most effective method for retaining the nutritional and bioactive compounds in leaves. Research shows leaves dried in shade possess significantly higher concentrations of flavonoids, saponins and tannins compared to those dried under direct sunlight [12]. Among various methods, shade-dried Moringa leaves were reported to have the highest flavonoid levels [13]. Additionally, shade-dried Moringa leaves showed higher carbohydrate (43.03 g/100 g) and ascorbic acid content (155.80 mg/100 g) compared to other drying methods [14]. This method also excels in preserving essential oils and the natural colour of herbs better than hot-air drying, sun drying, microwave drying and freeze-drying techniques [15]. Importantly, shade-dried products have been found to maintain their quality with a shelf life extending up to one year [16].

Therefore, this study aims to evaluate total antioxidant activity and to comprehensively identify the antioxidant compounds present in the methanolic extract of *Moringa oleifera* dried leaf powder using the Liquid Chromatography-Electro spray Ionization-Tandem Mass Spectrometry (LC-ESI-MS) technique for detailed phytochemical analysis. While conventional mass spectrometry methods typically provide information on groups of antioxidant compounds, LC-ESI-MS offers the advantage of detecting and characterizing individual antioxidant molecules with greater specificity [5, 17, 18]. Therefore, LC-ESI-MS has been selected for the detailed profiling of antioxidant constituents in this research.

Materials and method

Chemicals

HPLC-grade methanol (filtered through 0.2 micron membrane), 1,1-diphenyl-2-picrylhydrazyl (DPPH).

Collection and Processing of Plant Material

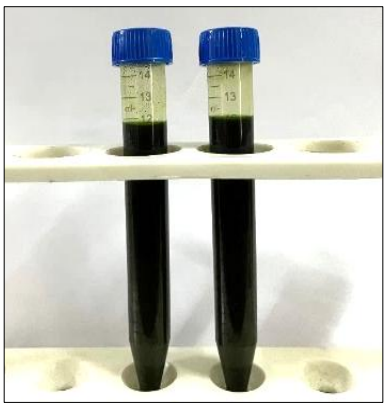
The leaves of *Moringa oleifera* were collected from matured healthy tree from Serampore, North 24 Parganas district, West Bengal, India; during the month of March to April, 2025. The leaves were separated from branches, sorted and washed with sterile water to eliminate dirt and debris, then shade-dried at room temperature (30°C) for 5 days to preserve their quality for further analysis. The dried leaves were finely powdered using a mixer grinder for further analysis. Larger particles were removed by sieving (60 microns diameter) using a Brass Frame Test Sieve (Brand: LABLINE INDIA). The resulting fine powder was stored in airtight zip-lock polythene bags for further studies [19].

Fig 1: Shade-dried leaves of *Moringa oleifera*

Fig 2: Dried leaf powder of *Moringa oleifera*

Preparation of Plant Extract

2 g of *Moringa oleifera* dried leaf powder was extracted with 10 mL of HPLC-grade methanol and kept at room temperature (30°C) for 72 hours [17]. After desired period of incubation, the mixture was centrifuged at 10,000 rpm for 15 minutes to separate solid residues [5, 17]. The clear supernatant was carefully collected and stored at refrigerated conditions as the stock solution of the plant extract, with a concentration of 200 mg/mL, for future analysis (Figure 3).



a

b

Fig 3: Dried leaf powder extract with HPLC-grade methanol; a - before and b – after centrifugation

Qualitative and Quantitative Analysis of Phytochemicals

Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry (LC-ESI-MS) Analysis of Phytochemicals: For LC-ESI-MS analysis, 0.1 mL of the stock solution of the plant extract was diluted with 9.9 mL of HPLC-grade methanol, resulting in a solution with a final concentration of 2 mg/mL, suitable for instrumental detection and profiling (Figure 4). This dilution was necessary to ensure better results in the DPPH assay and LC-ESI-MS analysis and to avoid any noise in the sample. The dilute solutions were filtered using a syringe filter (nylon membrane disc with 0.22 µm porosity, 25 mm diameter; AANIJ brand) to remove impurities. Subsequently, 5 mL of the filtered solution was collected for analysis.

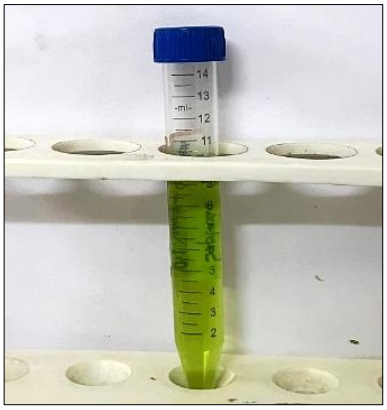


Fig 4: Dilute clear methanolic extract of *Moringa oleifera* dried leaf powder

Mass spectrometric profiling of phytochemicals was performed using a XEVO G2-XS QT of LC-MS system equipped with an electrospray ionization (ESI) source in direct infusion mode. A 2 mL vial containing the sample (dilution: 2 mg/mL) was manually placed, while the system operated automatically. The ESI conditions were optimized with a source capillary voltage of 3.5 kV, source temperature set at 100°C and a de-solvation temperature of 300°C. Cone gas and de-solvation gas flow rates were maintained at 49 L/hr and 398 L/hr respectively, to ensure optimal ionization [17].

Phytochemicals were identified by plotting the percentage of each compound (y-axis) against its mass-to-charge ratio (m/z) (x-axis) and comparing the spectral data with authenticated standards. Identification was further confirmed by matching the spectra with entries from the Mass Bank database. Only those compounds that showed a confirmed match were considered and included in the study [17].

The analysis employed Electrospray Ionization in both positive and negative modes across multiple m/z ranges for compound detection: ES+MS and ES−MS 100–500, 500–1000, 100–2000, 1000–1500 and 1500–2000. This comprehensive approach allowed precise identification of phytochemicals while minimizing background interference, ensuring the accuracy and reliability of the results. By covering an extended mass range and utilizing dual ionization modes, the method ensured robust, accurate, and reliable phytochemical profiling, capturing both low and high molecular weight compounds effectively.

DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity: The free radical scavenging capacity of the plant extracts was determined using DPPH assay [20, 21]. In this method, the free radical scavenging activity (RSA) of the sample was tested using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. To prepare the DPPH stock solution, 4 mg of DPPH was dissolved in 100 mL of methanol, which has a concentration of 0.04 mg/mL. This solution showed an absorbance of approximately 0.973 at 517 nm, making it appropriate for antioxidant activity evaluation. For the assay, 3 mL of this DPPH workable solution was mixed with 25 µL, 50 µL and 100 µL of the dilute clear plant extract (concentration: 2 mg/mL or 0.002 g/mL) in three separate test tubes. As a control, 3 mL of DPPH solution was combined with 100 µL methanol and incubated in complete darkness for 30 minutes before analysis. After incubation, absorbance was recorded at 517 nm using a Lasany LI 295 UV-Visible Spectrophotometer (wavelength range: 190–1100 nm; 1 nm diffraction grating) [21]. Radical Scavenging Activity (RSA) percentage was determined using a standard formula by evaluating absorbance changes before and after sample treatment [22];

Radical Scavenging Activity (%) or Antioxidant Activity (%)

$$= \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \text{ x } 100$$

The antioxidant potential of the studied extracts was evaluated by determining their IC_{50} values, which represent the concentration required to reduce DPPH radical levels by 50% [23]. A lower IC_{50} value signifies greater radical scavenging ability, indicating stronger antioxidant potential of the sample [24]. To determine the IC_{50}, a graph was plotted with radical scavenging activity (%) on the Y-axis and extract concentration (mg/mL) on the X-axis. The regression equation obtained from the graph was used to identify the values of each parameter and calculate the IC_{50} value, indicating the concentration required to inhibit 50% of DPPH radicals. The graph followed the linear equation $y = mx + c$, based on which the values of each parameter were identified. The slope (m) and intercept (c) were determined from the graph. Using these values, the IC_{50} was then calculated using the standard formula provided below [22];

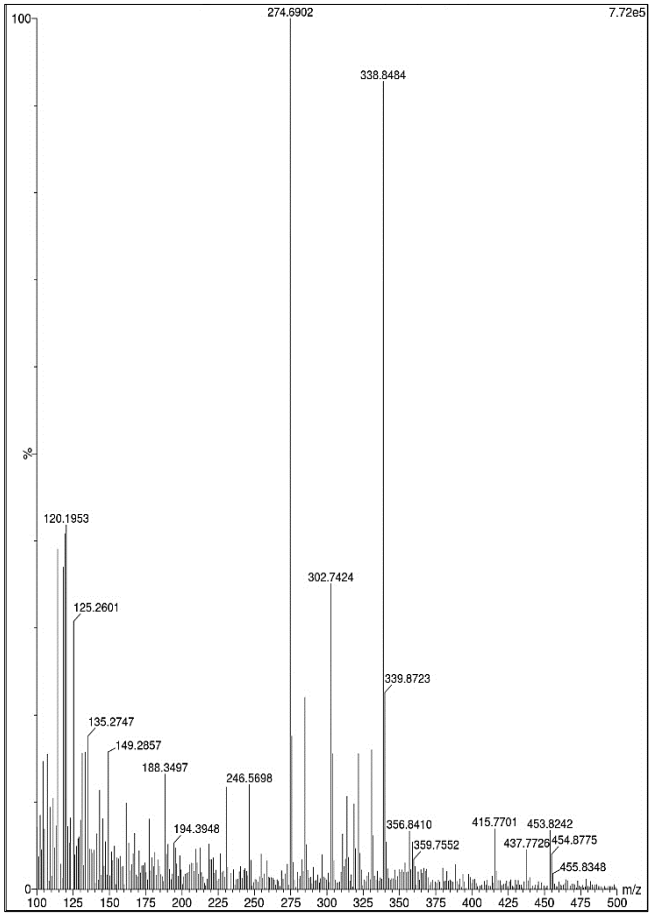
$$y = mx + c$$

$$IC_{50} = \frac{(y - c)}{m} = \frac{(50 - c)}{m}$$

This method provides a quantitative assessment of antioxidant efficiency. A lower IC_{50} value thus signifies a greater antioxidant potential, demonstrating the extract's enhanced ability to scavenge free radicals effectively [19].

Result and discussion

Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry (LC-ESI-MS) Analysis of Phytochemicals: The phytochemicals of the methanolic extract of *Moringa oleifera* dried leaves was analysed using the LC-ESI-MS method. Figure 5 shows the LC-ESI-MS profile, analysed under Electrospray Ionization in both Positive and Negative Modes across multiple m/z ranges: 100–500, 500–1000, 100–2000, 1000–1500, and 1500–2000. By comparing the results with authentic standards, total 67 phytochemicals were identified detailed in Table 1, Table 2 and Table 3, with Rutine, Naringenin, Demethyl medicarpin, 4'-O-(2'-E-Coumaroyl GluA) (1-2) GluA) Apigenin, Malvidin, 10-deacetylbaccatin (10-DAB) and Lobeline are found to be the most abundant group within the extract.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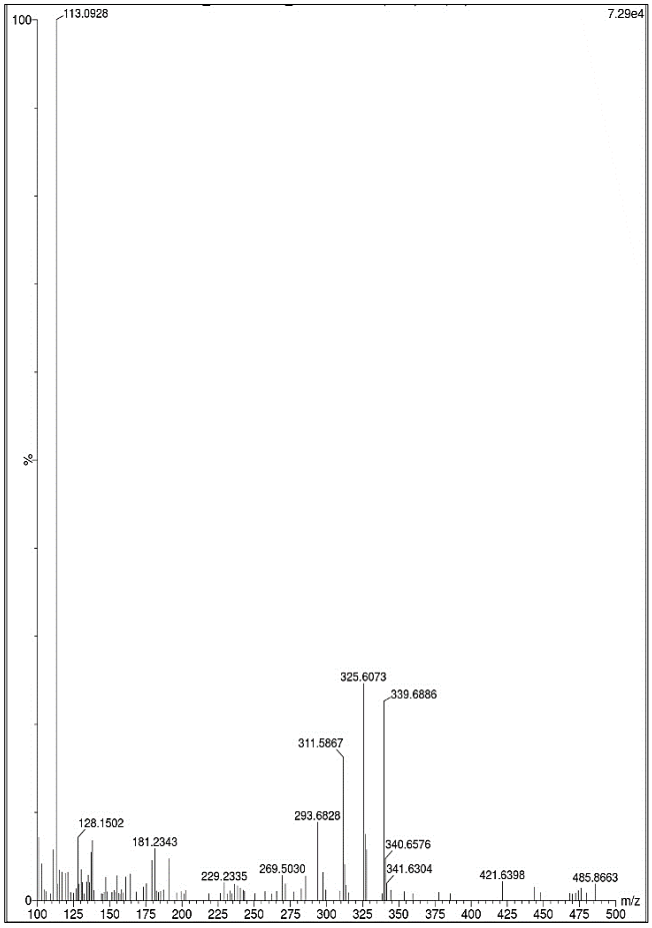
%
m/z

a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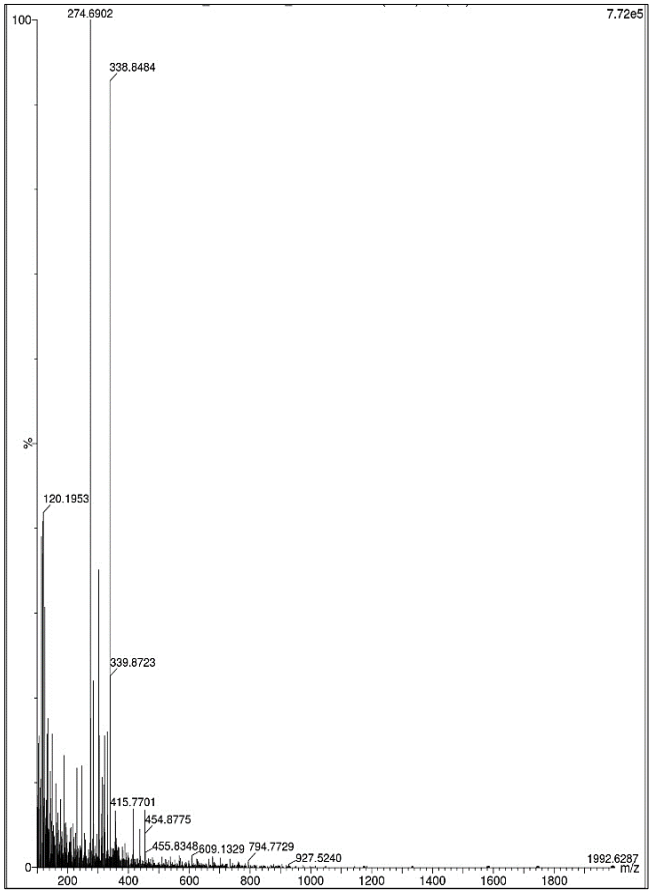
b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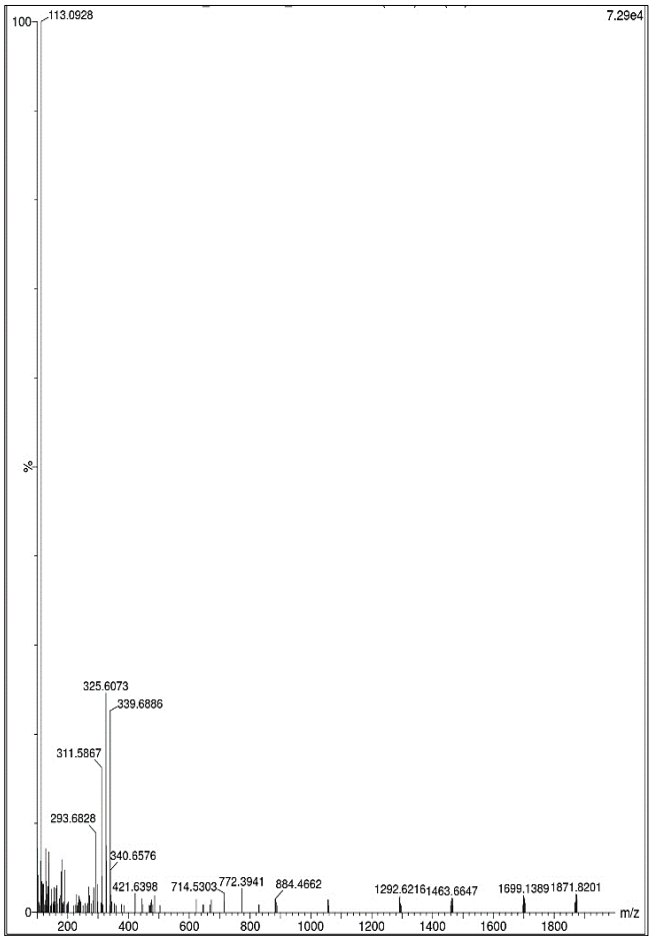
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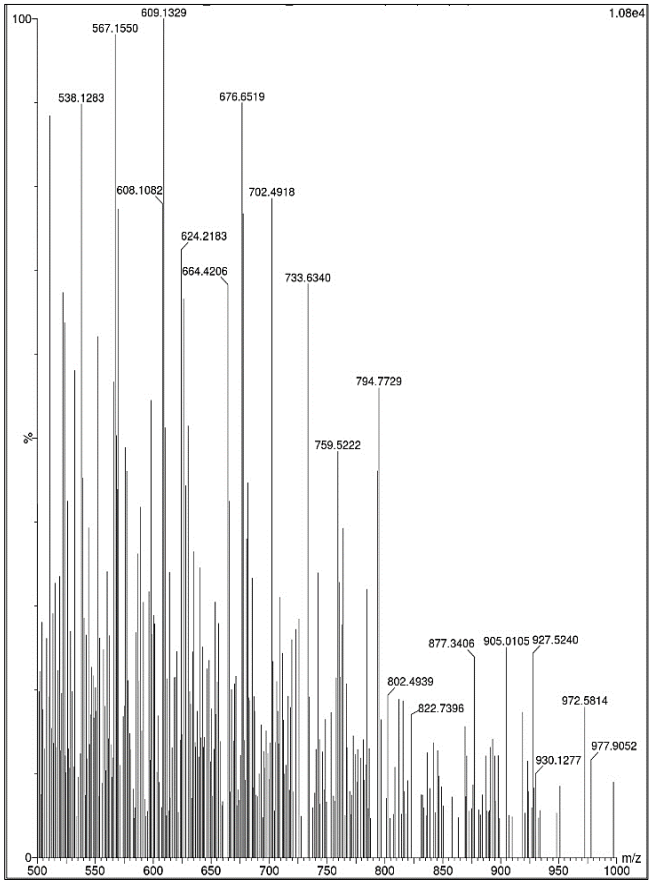
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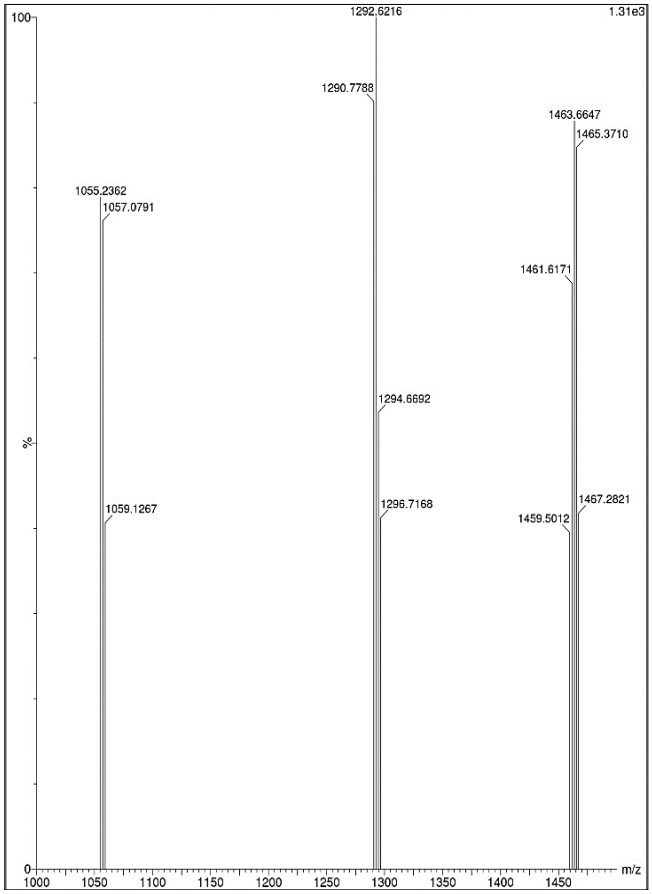
m/z

c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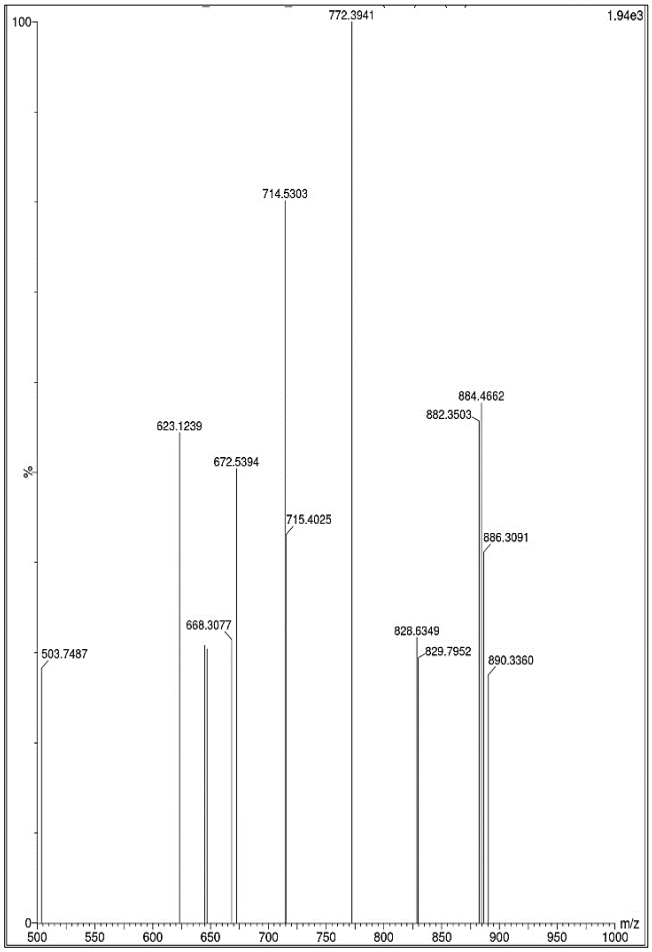
m/z

d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m/z

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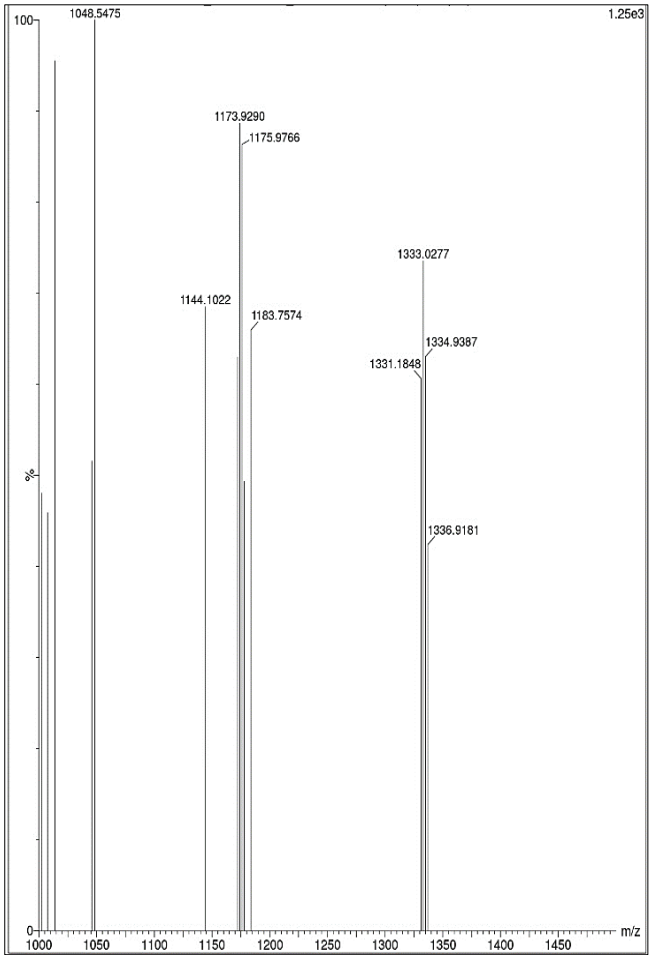
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%
m/z

i

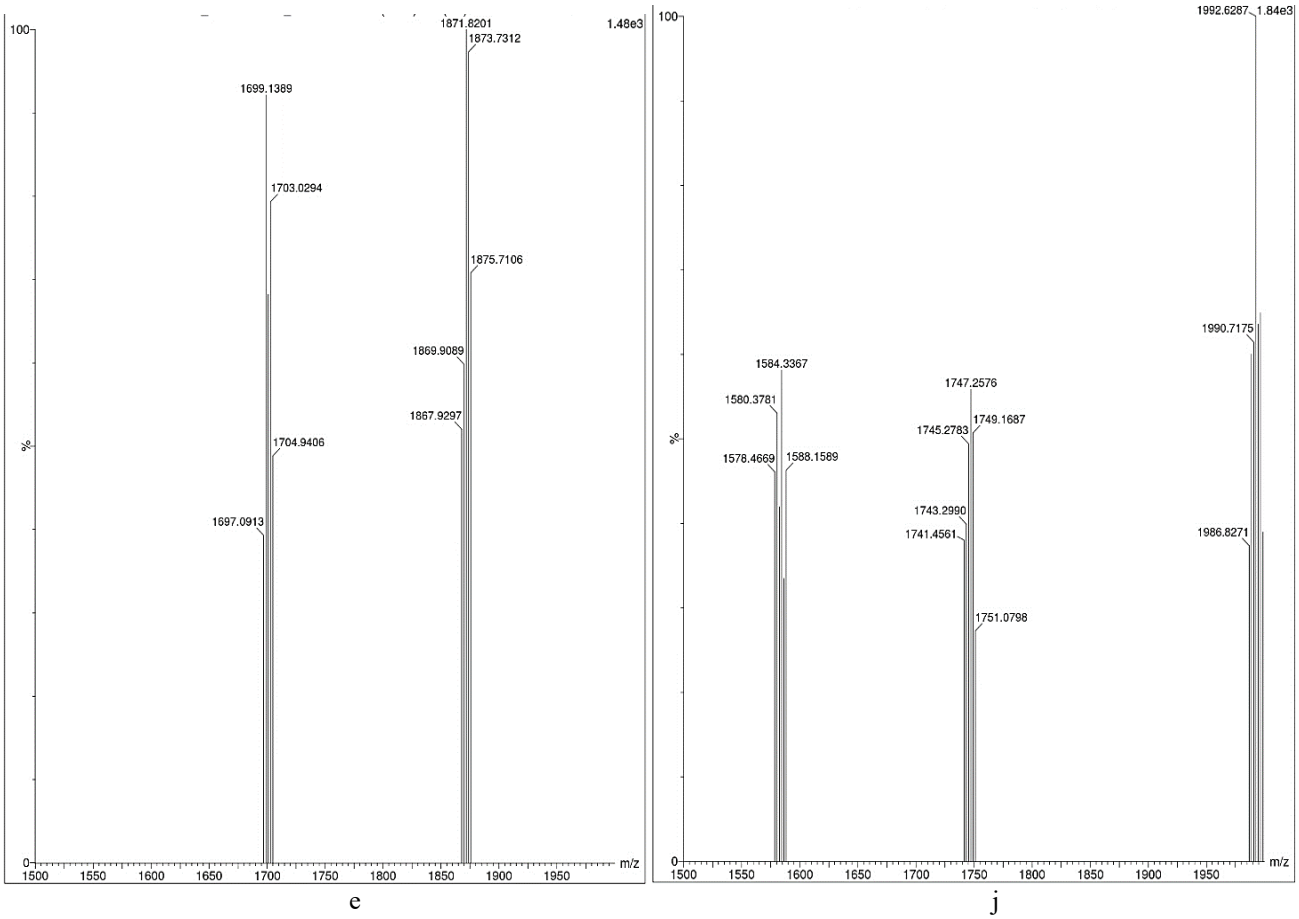
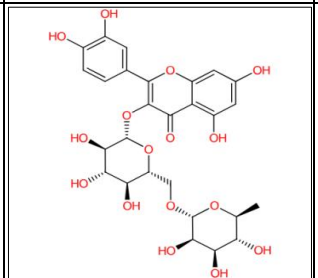
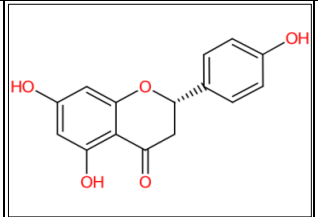
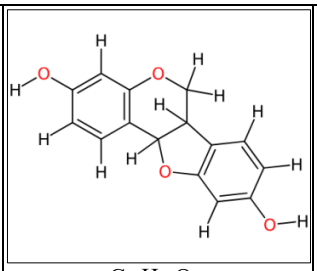
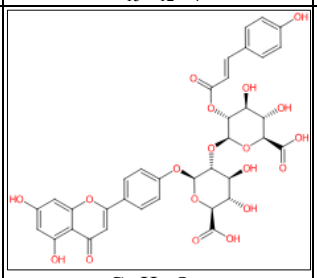
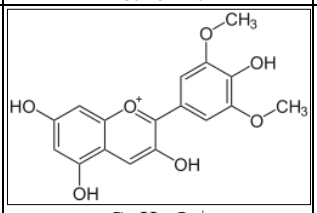
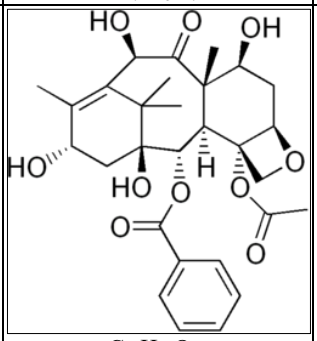
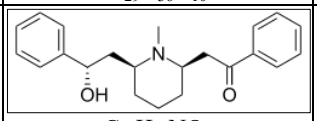
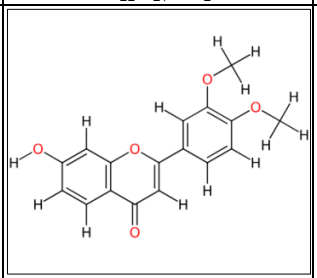
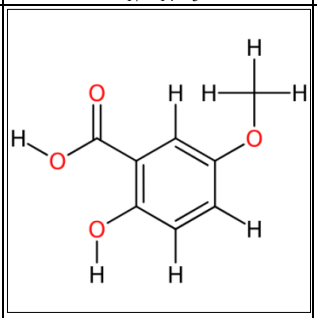


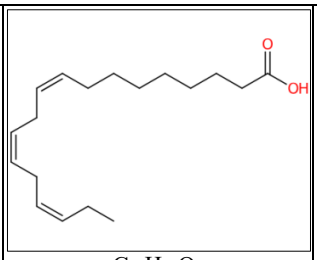
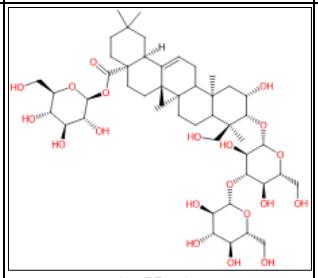
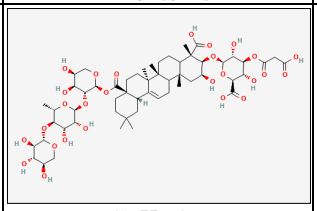
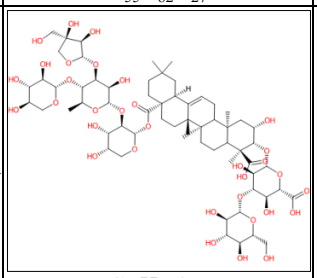
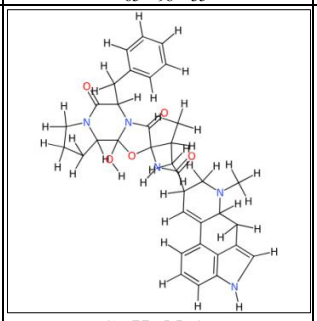
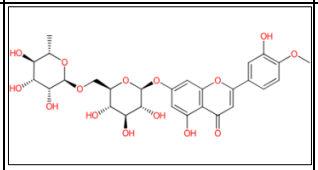
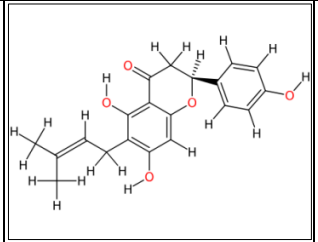
Fig 5: LC-ESI-MS Profiles of *Moringa oleifera* Leaf Extract Acquired Under Multiple Electrospray Ionization Modes Across Different m/z Ranges:(a–e) Positive Ion Mode: 100–500 (a), 100–2000 (b), 500–1000 (c), 1000–1500 (d), 1500–2000 (e). (f–j) Negative Ion Mode: 100–500 (f), 100–2000 (g), 500–1000 (h), 1000–1500 (i), 1500–2000 (j).

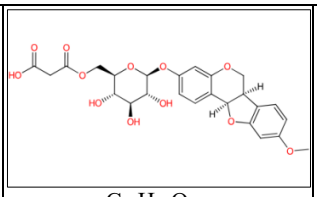
Spectral data were compared with authenticated standards and validated using Mass Bank entries. Only compounds with confirmed spectral matches were selected, leading to the identification of the phytochemicals in the methanolic extract through this rigorous verification process. The identified phytochemicals have been classified into three separate tables based on their relative intensity values. The term "Relative Intensity (% of Phytochemicals)" refers to the strength of the mass spectrometric signal detected for a specific compound, expressed as a percentage relative to the most intense peak in the spectrum, which is set at 100%. Phytochemicals listed in Table 1 exhibit the highest relative intensity, ranging from 75% to 100%. Those in Table 2 show moderate relative intensity, between 50% and 74%. Finally, Table 3 includes phytochemicals with the lowest relative intensity, ranging from 25% to 49%.

Table 1: High-Intensity Phytochemicals (75–100%) Identified in Methanolic Extract of *Moringa oleifera* leaf via LC-ESI-MS Analysis

Sl. No.	Phytochemicals	Structure of Phytochemicals & Molecular formula	Molecular Mass (g/mol)	Peak data (m/z ratio)	Relative Intensity % (% Of Phytochemicals)	Score
1	Rutine	 $C_{27}H_{30}O_{16}$	610.15338	609.1329 (ES+)	100	0.9999
2	Naringenin	 $C_{15}H_{12}O_5$	272.25601	274.6902 (ES+)	100	0.3951

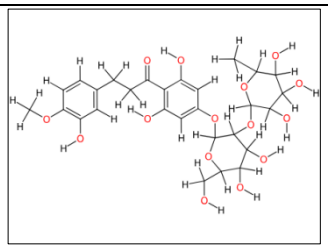
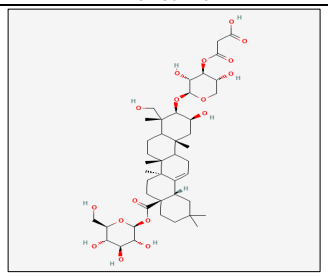
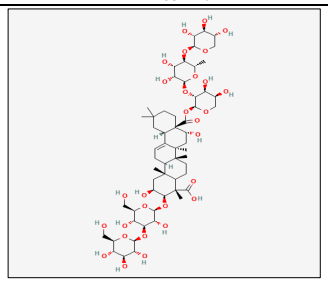
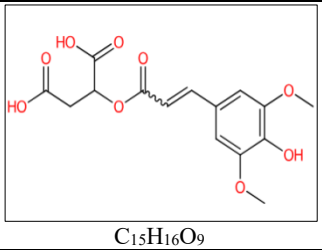
3	Demethyl medicarpin	 $C_{15}H_{12}O_4$	256.07361	113.0928 (ES-)	100	0.173
4	4'-O-(2'-E-Coumaroyl GluA) (1-2) GluA) Apigenin	 $C_{36}H_{32}O_{19}$	768.1538	772.3941 (ES-)	100	0.1195
5	Malvidin	 $C_{17}H_{15}O_7^+$	331.2968	1048.5475 (ES+)	100	0.0911
6	10-deacetylbaccatin (10-DAB)	 $C_{29}H_{36}O_{10}$	544.59	567.155 (ES+)	99	0.7778
7	Lobeline	 $C_{22}H_{27}NO_2$	337.463	338.8484 (ES+)	94	0.9883
8	3',4'-Dimethoxy-7-hydroxyflavone	 $C_{17}H_{14}O_5$	298.08411	624.2183 (ES+)	90	0.3795
9	5-Methoxysalicylic acid	 $C_8H_8O_4$	168.04230	538.1283 (ES+)	90	0.1917

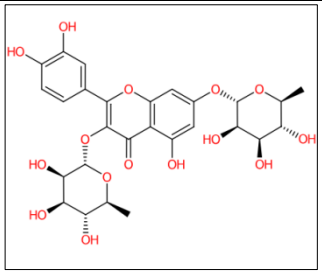
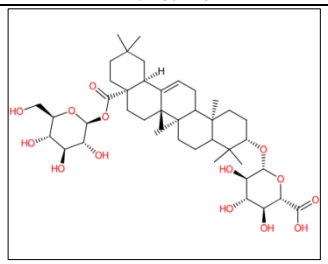
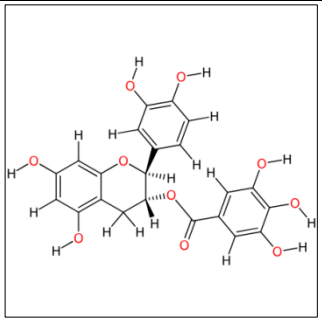
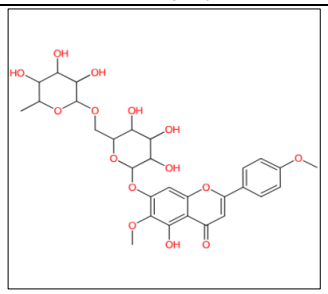
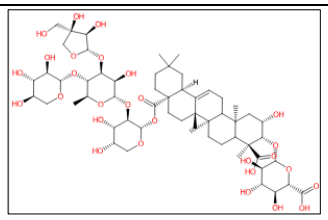
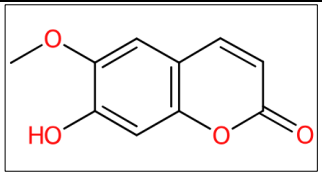
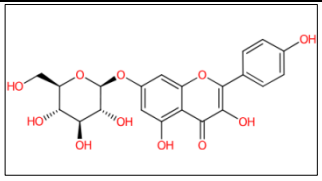
10	a-Linolenic acid	 $C_{18}H_{30}O_2$	278.22461	1173.929 (ES+)	90	0.0879
11	3-Glu (1,3) Glu-28-Glu Bayogenin	 $C_{48}H_{78}O_{20}$	974.5086	1463.6647 (ES-)	89	0.1233
12	3-((3'-Malonyl) GlcA)-28-Xyl (1-4) Rha (1-2) Ara Medicagenic acid	 $C_{55}H_{82}O_{27}$	1175.2	1175.9766 (ES+)	88	0.036
13	3-Glu (1-3) GluA-28-Xyl (1-4) [Api (1-3)] Rha (1-2) Ara Medicagenic acid	 $C_{63}H_{98}O_{33}$	1382.59900	714.5303 (ES-)	81	0.1054
14	Ergocristine	 $C_{35}H_{39}N_5O_5$	609.29510	608.1082 (ES+)	79	0.9999
15	Diosmin	 $C_{28}H_{32}O_{15}$	608.17413	676.6519 (ES+)	79	0.1125
16	6-Prenylnaringenin	 $C_{20}H_{20}O_5$	340.13110	702.4918 (ES+)	79	0.08

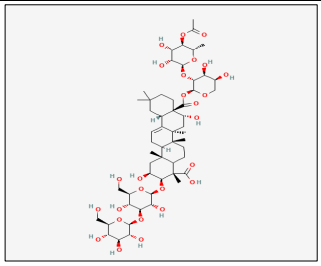
17	6'-Malonyl-3-Glu Medicarpin	 $C_{25}H_{26}O_{12}$	518.14240	1057.0791 (ES-)	78	0.2075

In the above table (Table. 1), we have listed 17 phytochemicals with relative intensities ranging from 75% to 100%. Very few studies have conducted LC-ESI-MS profiling specifically on *Moringa oleifera* leaf extracts, making our findings particularly significant. Among the identified compounds, some have already been reported in previous research works. However, we have also detected several phytochemicals that, to the best of our knowledge, have not been previously reported in *Moringa oleifera* leaf extract. These newly identified compounds include Demethyl medicarpin, 4'-O-(2'-E-Coumaroyl GluA) (1→2) GluA, Apigenin, Malvidin, 10-Deacetylbaccatin (10-DAB), Lobeline, 3',4'-Dimethoxy-7-hydroxyflavone, 5-Methoxysalicylic acid, 3-Glu (1→3) Glu-28-Glu Bayogenin, 3-Glu (1→3) GluA-28-Xyl (1→4) [Api (1→3)] Rha(1→2) Ara Medicagenic acid, Ergocristine, Diosmin, 6-Prenylnaringenin, and 6'-Malonyl-3-Glu Medicarpin. The detection of these compounds highlights the potential of *Moringa oleifera* leaves as a rich and underexplored source of bioactive phytochemicals.

Table 2: Moderate-Intensity Phytochemicals (50–74%) Identified in Methanolic Extract of *Moringa oleifera* leaf via LC-ESI-MS Analysis

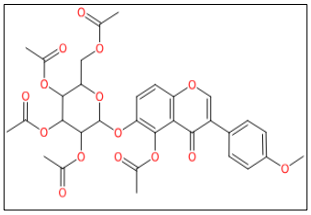
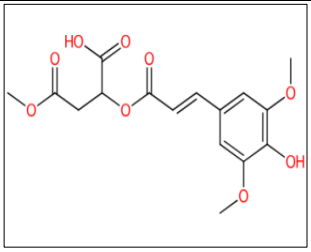
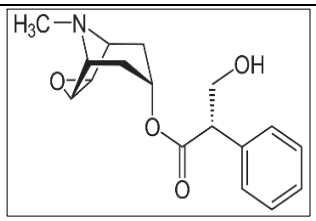
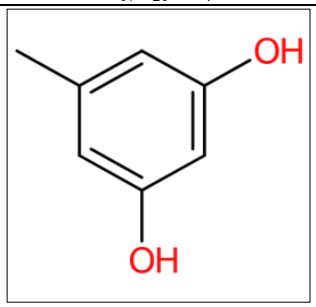
Sl. No.	Phytochemicals	Structure of Phytochemicals & Molecular formula	Molecular Mass (g/mol)	Peak data (m/z ratio)	Relative Intensity % (% Of Phytochemicals)	Score
1	Hex-hexA-dhex-Pen-Pen-Pen (or hex-hex-hex-hex-dhex-Mal) Zanhic acid (PUT)	- $C_{70}H_{94}O_{29}$	1398.58813	1333.0277 (ES+)	74	0.118
2	Neohesperidin dihydrochalcone	 $C_{28}H_{36}O_{15}$	612.20538	664.4206 (ES+)	70	0.1032
3	3-((3'-Malonyl) Xyl)-28-Glu Bayogenin	 $C_{44}H_{68}O_{17}$	869.0	733.634 (ES+)	69	0.0744
4	3-GluA-28-Xyl (1-4) Rha (1-2) Ara Zanhic acid	 $C_{58}H_{92}O_{29}$	1253.3	1183.7574 (ES+)	68	0.0749
5	Sinapoyl malate	 $C_{15}H_{16}O_9$	340.28400	1869.9089 (ES-)	61	0.13

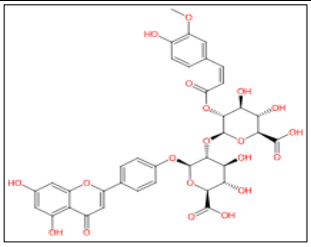
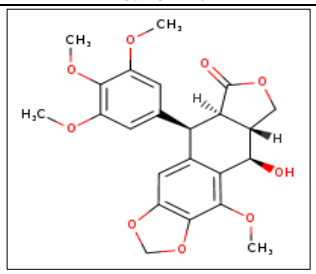
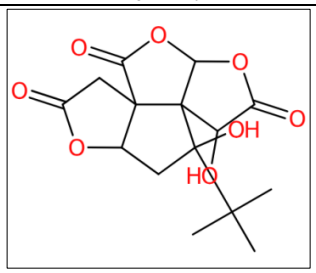
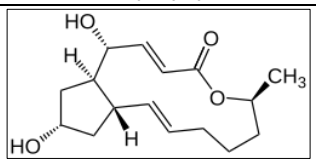
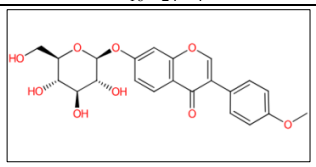
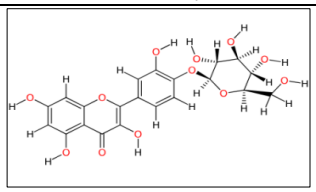
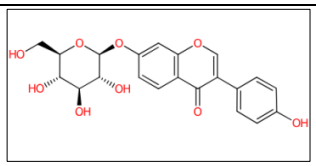
6	3-Rha-7-Rha Quercetin	 $C_{27}H_{30}O_{15}$	594.15851	1584.3367 (ES+)	60	0.0586
7	3-GlcA-28-Glc oleanolic acid	 $C_{42}H_{66}O_{14}$	794.44531	794.7729 (ES+)	57	0.3008
8	Catechin gallate	 $C_{22}H_{18}O_{10}$	442.09000	884.4662 (ES-)	56	0.4082
9	Pectolinarin	 $C_{29}H_{34}O_{15}$	622.18976	623.1239 (ES-)	54	0.8017
10	3-(Glc (1-2) Glc (1-2) Glc)-28-(Xyl (1-4) [Ara (1-3)] Rha (1-2) Ara Zanhic acid	-	-	1580.3781 (ES+)	54	0.0694
11	3-GluA-28-Xyl (1-4) [Api (1-3)] Rha (1-2) Ara Medicagenic acid	 $C_{57}H_{88}O_{28}$	1220.5462	1294.6692 (ES-)	53	0.0596
12	Scopoletin	 $C_{10}H_{8}O_{4}$	192.17000	120.1953 (ES+)	53	0.0327
13	Kaempferol-7-O-glucoside	 $C_{21}H_{20}O_{11}$	448.10101	672.5394 (ES-)	51	0.0986

14	3-(Glu (1-3) Glu)-28-(Rha(4-O-acetyl) (1-2) Ara) Zanhic acid	 $C_{55}H_{86}O_{26}$	1163.3	1745.2783 (ES+)	50	0.1529

In the above table (Table. 2), we have listed 14 phytochemicals with relative intensities ranging from 50% to 74%. While some of these compounds have been previously reported in earlier studies, our analysis also revealed several phytochemicals that, to the best of our knowledge, have not been documented before in *Moringa oleifera* leaf extracts. These newly identified compounds include Hex-hexA-dhex-Pen-Pen-Pen (or Hex-Hex-Hex-Hex-dHex-Mal) Zanhic acid (PUT), Neohesperidin dihydrochalcone, 3-[(3'-Malonyl)Xyl]-28-Glu Bayogenin, 3-GluA-28-Xyl (1→4) Rha (1→2) Ara Zanhic acid, Sinapoyl malate, Pectolinarin, 3-[Glc(1→2) Glc(1→2) Glc]-28-(Xyl (1→4) [Ara (1→3)] Rha(1→2) Ara) Zanhic acid and 3-[Glu (1→3) Glu]-28-[Rha (4-O-acetyl) (1→2) Ara] Zanhic acid. The identification of these compound points to the phytochemical richness of *Moringa oleifera* leaves and emphasizes their potential as a valuable yet underexplored source of diverse bioactive molecules.

Table 3: Low-Intensity Phytochemicals (25–49%) Identified in Methanolic Extract of *Moringa oleifera* leaf via LC-ESI-MS Analysis

Sl. No.	Phytochemicals	Structure of Phytochemicals & Molecular formula	Molecular Mass (g/mol)	Peak data (m/z ratio)	Relative Intensity % (% Of Phytochemicals)	Score
1	{3,4,5-triacetyloxy-6-[5-acetyloxy-3-(4-methoxyphenyl)-4-oxochromen-6-yloxy]-2 H-3,4,5,6-tetrahydropyran-2-yl} methyl acetate	 $C_{32}H_{32}O_{15}$	656.17412	715.4025 (ES-)	45	0.9999
2	Sinapoyl malate-4'-methyl ester	 $C_{16}H_{18}O_9$	354.09509	1059.1267 (ES-)	42	0.0737
3	Hex-hex-hexA-hederagenin (or hex-hex-hexA-2-hydroxyoleanolic acid)	$C_{55}H_{72}O_{15}$	972.48712	1459.5012 (ES-)	40	0.1571
4	Scopolamine	 $C_{17}H_{21}NO_4$	303.358	302.7424 (ES+)	35	0.9999
5	Orcinol	 $C_7H_8O_2$	124.05243	125.2601 (ES+)	33	0.9999

6	4'-O-(2'-Z-Feruloyl GluA (1-2) GluA) Apigenin	 $C_{37}H_{34}O_{20}$	798.1643	829.7952 (ES-)	32	0.1226
7	5-methoxypodophyllotoxin	 $C_{23}H_{24}O_9$	444.436	503.7487 (ES-)	30	0.9999
8	Bilobalide	 $C_{15}H_{18}O_8$	326.10016	325.6073 (ES-)	27	0.9999
9	Brefeldin A	 $C_{16}H_{24}O_4$	280.36	339.6886 (ES-)	26	0.9999
10	Formononetin-7-O-glucoside (Ononin)	 $C_{22}H_{22}O_9$	430.12640	905.0105 (ES+)	26	0.4798
11	Spiraeoside	 C21H20O12	464.09549	927.524 (ES+)	26	0.4063
12	Daidzin	 $C_{21}H_{20}O_9$	416.11072	877.3406 (ES+)	26	0.2927

The Table 3 lists 12 phytochemicals with relative intensities between 25% and 49%. While several of these compounds have been previously reported in *Moringa oleifera* leaf extracts, our LC-ESI-MS analysis also identified a number of novel compounds not documented earlier. These include{3,4,5-triacetyloxy-6-[5-acetyloxy-3-(4-methoxyphenyl)-4-oxochromen-6-yloxy]-2H-3,4,5,6-tetrahydropyran-2-yl} methyl acetate, Hex-hex-hexA-hederagenin, Scopolamine, Orcinol, 4'-O-(2'-Z-Feruloyl GluA (1-2) GluA) Apigenin, 5-methoxypodophyllotoxin, Bilobalide, Brefeldin A and Daidzin.

Across the above three Tables (Table.1; Table.2 and Table. 3), a total of 43 phytochemicals have been listed along with their respective details. Each compound is annotated with the ionization mode detected through the LC-ESI-MS method: denoted as ES^+ for positive ionization mode and ES^- for negative ionization mode. In some cases, a few compounds were detected at multiple mass-to-charge (m/z) ratios. For such instances, we have included the m/z value corresponding to the highest relative intensity in the table.

In addition to the 43 identified phytochemicals, 24 other compounds were also detected in very trace amounts, and

hence their detailed profiles have not been included. However, their names and corresponding relative intensities are listed here: 4'-O-(2'-E-Feruloyl GluA (1-2) GluA) Apigenin (20%), 2,6-Dihydroxyacetophenone (19%), Nebularine (18%), Hex-hex-hexA-hederagenin (18%), Kynurenic Acid (17%), Glutamic acid (16%), Asiatic acid (13%), Epigallocatechin gallate (11%), Emodin (10%), Harman (8%), Lagochiline (7%), Canadine (7%), Daphnoretine Acetate (6%), Salsoline (6%), Thiodicarb (6%), Foramsulfuron (5%), Inosine (4%), (+)-(4,6-O-Benzylidene) methyl-α-D-glucopyranoside (4%), Rosmarinic acid (4%), 1,9-dideoxyforskolin (4%), Benzo-diazepinone derivative (3%), Hydrocortisone (3%), 3-Glu-3,4',7-trihydroxyisoflavanone (3%), and Resveratrol (2%).

The methanolic extract of *Moringa oleifera* leaves revealed a remarkable diversity of phytochemicals, with 67 compounds identified using authentic standards. Our study discovered several previously unreported compounds. Newly identified compounds were characterized using their mass-to-charge ratios and relative intensities, providing valuable insights into their molecular properties and potential bioactivity.

The LC-ESI-MS analysis identified a diverse range of bioactive metabolites, including phenolic acids, flavonoids, alkaloids, and glycosylated derivatives, reflecting the rich chemical diversity of the plant. In several cases, the same phytochemical appeared at different mass-to-charge (m/z) ratios due to fragmentation during LC-ESI-MS analyse which is a common phenomenon in mass spectrometry that produces multiple ions from a single compound [25]. To maintain clarity, we have listed the m/z value corresponding to the highest relative intensity for such compounds in the table. These fragmentation patterns are essential for the accurate identification and structural characterization of phytochemicals. Furthermore, variations in m/z values often result in differing relative intensities, emphasizing the analytical complexity of mass spectrometry and the importance of careful interpretation for reliable phytochemical profiling.

Notably, the most abundant compounds included Rutine, Naringenin, Malvidin, Demethyl medicarpin, 4'-O-(2'-E-Coumaroyl GluA) (1-2) GluA) Apigenin, and 10-deacetylbaccatin, each exhibiting relative intensities of 99–100%. Additionally, Lobeline (93–94%), α-Linolenic acid (90%), Catechin gallate (89%) and 3-Glu (1,3) Glu-28-Glu Bayogenin (89%) also showed high ionization responses. The presence of Rutine and Naringenin, both known flavonoids with strong antioxidant properties, suggests their significant contribution to the bioactivity of the extract. These findings support subsequent results of the study, which emphasized the identified compounds for their significant free radical scavenging and antioxidant potential. The high intensity values highlight their abundance and potential pharmacological relevance, supporting the use of *Moringa oleifera* in traditional and functional applications.

The presence of quinic acid derivatives and various glycosides, including complex saponins such as medicagenic acid conjugates and bayogenin derivatives, indicates extensive metabolic diversity. Compounds like quercetin and its glycosylated forms, commonly found in Moringa, are known for their anticancer, anti-inflammatory and antioxidant effects [5]. Interestingly, the detection of demethyl medicarpin and formononetin glucosides adds to the known iso-flavonoid content of the plant, supporting its use in traditional medicine systems. The varying molecular weights and ion intensities among compounds suggest differential extractability and ionization efficiency, likely influenced by compound polarity and structure. Additionally, the presence of multiple adducts and isomers of specific compounds such as catechin, gallates and brefeldin A underscores the complexity of the plant's secondary metabolite profile.

Compared to previous studies, this research has identified a substantially higher number of compounds. Previous studies reported 18 compounds in one study [17], 15 in another [5], and 37 in a separate study [26], all identified from methanolic extracts of *Moringa oleifera* leaves. While fewer compounds were identified in these studies, their structural details were also not extensively described. Our research provides a more comprehensive metabolite profile, reflecting advancements in detection techniques and analytical precision. Environmental factors, including geographical location, climatic conditions, and harvest timing, are known to significantly influence phytochemical composition. These variables may explain the observed differences in compound abundance across various studies. The high intensity values of key compounds reinforce their relevance in bioactivity, particularly in antioxidant mechanisms, free radical scavenging and potential pharmacological applications.

DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Activity: DPPH radical scavenging assay is widely used to assess antioxidant activity of plant extracts, which expresses the results as IC_{50} values (mg/mL). IC_{50} refers to the concentration of extract required to inhibit 50% of DPPH radicals. A lower IC_{50} value corresponds to higher antioxidant activity, indicating a stronger free radical scavenging ability. The DPPH assay has been widely adopted in studies investigating antioxidant capacities of plant-derived compounds. In this method, the deep purple DPPH radical (picrylhydrazyl) is reduced by antioxidants to a pale-yellow form (picrylhydrazine), as they donate electrons or hydrogen atoms to neutralize the radical. The concentration-dependent reduction causes discoloration, indicating the percentage of radical scavenging activity (%RSA) [23].

In the present study, methanolic extract of dried *Moringa oleifera* leaf powder was assessed for free radical scavenging activity using the DPPH assay method. The antioxidant potential was quantified in terms of % RSA and IC_{50} value, effectively indicating the efficacy of the extract in neutralizing free radicals.

Table 4: Different concentrations of the sample were calculated

Sl. No.	Volume of DPPH stock solution taken	Volume of sample added	Total reaction volume	Concentration of plant extract
1	3 mL	25 µL = 0.025 mL	3.025 mL	0.002 g/mL × (0.025mL / 3.025 mL) = 0.0000165 g/mL =0.0165 mg/mL
2	3 mL	50 µL = 0.05mL	3.050 mL	0.002 g/mL × (0.05mL / 3.050 mL) = 0.0000328 g/mL =0.0328 mg/mL
3	3 mL	100 µL = 0.10 mL	3.10 mL	0.002 g/mL × (0.1 mL / 3.1 mL) = 0.0000645 g/mL =0.0645 mg/mL

The absorbance values of the sample at various concentrations, along with the control (standard OD), were obtained experimentally and are presented in Table 5. Based on these values, the Radical Scavenging Activity (%) or

Antioxidant Activity (%) was calculated at each concentration. The summarized results in Table 5 provide a basis for analysing the antioxidant potential of the sample.

Table 5: Absorbance and %RSA of the Sample at Various Concentrations

Calculation of % Radical Scavenging Activity from DPPH Assay			
Absorbance Measurement Data			
Concentration (mg/mL)	**Control**	**Sample**	**%RSA**
0.0165	0.294	0.289	1.70
0.0328	0.294	0.280	4.76
0.0645	0.294	0.275	6.46

To evaluate the antioxidant potential of the extract, the percentage of radical scavenging activity (%RSA) was calculated at different concentrations using the absorbance values. These %RSA values were then used to plot a graph for the determination of IC_{50}, which represents the concentration of extract required to scavenge 50% of DPPH radicals. The graphical method allows for a more precise estimation of antioxidant capacity.

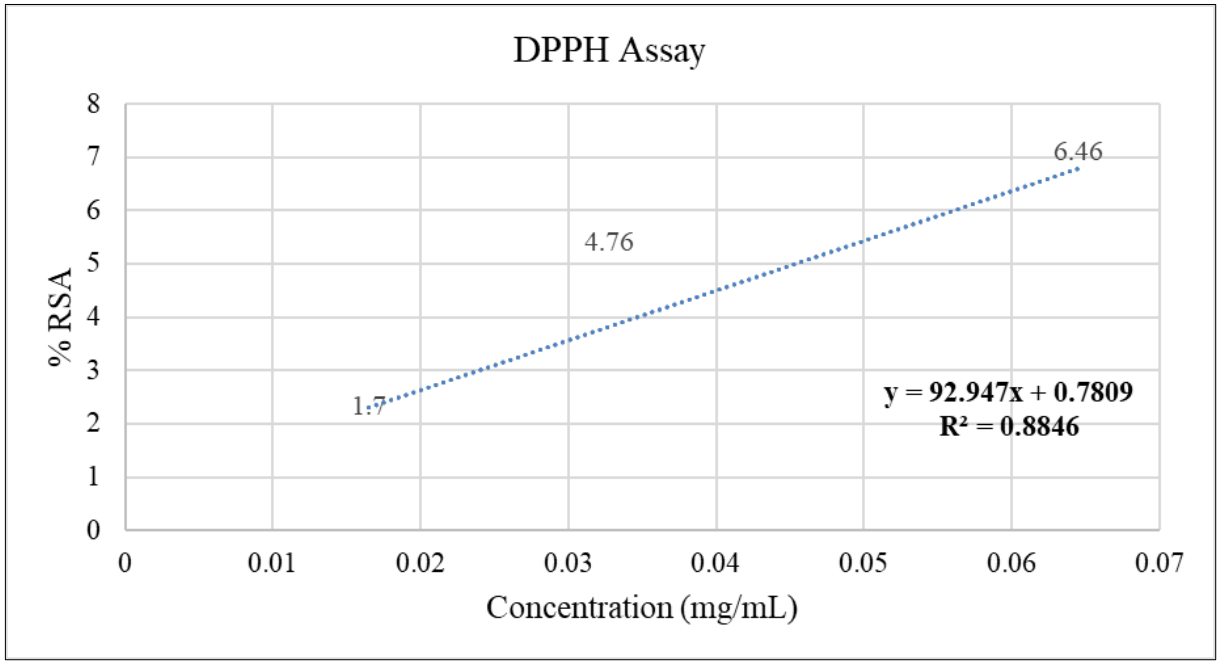


Fig 6: Linear Regression Plot of % Radical Scavenging Activity (%RSA) vs. Concentration (mg/mL) for Determination of IC_{50} value

The intercept (c) obtained from the graph was 0.7809 and the slope (m) was calculated to be 92.947. The IC_{50} value was calculated by applying the standard formula to the linear regression equation derived from the plot of radical scavenging activity (%) vs extract concentration (mg/mL).

$$y = mx + c$$

$$IC_{50} = \frac{(y - c)}{m} = \frac{(50 - c)}{m} = \frac{(50 - 0.7809)}{92.947} = 0.5295 \text{ mg/mL}$$

The IC_{50} of the methanolic extract of *Moringa oleifera* dried leaf powder was found to be 0.5295 mg/mL, which is relatively low. In another study [27], an IC_{50} value of 1.60 mg/mL was reported for DPPH assays using methanolic extracts of *Moringa oleifera* leaves. Similarly, [28] reported an IC_{50} value of 2.397 ± 0.10 mg/mL for methanolic extracts. In fact, some research works, such as that by [29], reported IC_{50} values of 0.4441mg/mL for the ethyl acetate extract and 0.71521 mg/mL for the n-hexane extract. When compared with these studies, the IC_{50} value of our methanolic extract sample is relatively lower, indicating that it possesses comparatively stronger antioxidant activity. As a lower IC_{50} value indicates higher antioxidant activity and stronger free radical scavenging ability, this suggests that our sample possesses strong antioxidant activity.

Conclusion

This study evaluated antioxidant potential via DPPH assay and identified the phytochemical profile of *Moringa oleifera* dried leaf powder using LC-ESI-MS analysis. A total of 67 bioactive compounds were identified, including flavonoids, phenolic acids, alkaloids, glycosides and saponins. Previously unreported compounds were characterized based on mass-to-charge ratios and relative intensities, highlighting the plant's metabolic diversity. 30 novel phytochemicals within the 25–100% relative intensity range, which have been reported for the first time in this study. The abundance of highly ionized metabolites, such as quinic acid derivatives, glycosylated flavonoids and iso-flavonoids, further emphasizes its pharmacological significance. A total of 67 bioactive compounds were identified, including flavonoids, phenolic acids, alkaloids, glycosides and saponins. Thirty novel phytochemicals with relative intensities ranging from 25% to 100% are reported here for the first time, based on their mass-to-charge ratios and intensities. This highlights the plant's rich metabolic diversity. The high abundance of ionized metabolites, such as quinic acid derivatives, glycosylated flavonoids, and iso-flavonoids, further highlights the pharmacological potential of *Moringa oleifera* leaf extracts. Antioxidant evaluation using DPPH assay revealed an IC_{50} value of 0.5295 mg/mL, demonstrating strong radical

scavenging activity. Comparisons with prior research suggest that *M. oleifera* exhibits superior antioxidant potential, reinforcing its medicinal and nutraceutical applications. Environmental factors, such as geographical location and climate, may influence compound variation, explaining discrepancies with earlier studies. Overall, this research enhances understanding of *M. oleifera*'s phytochemical complexity, validating its role in functional foods and therapeutic formulations while encouraging further exploration of its bioactive properties.

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