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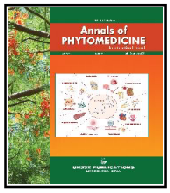

Original Article : Open Access

Proximate analysis and phytochemical characterization of methanolic extract of *Moringa oleifera* Lam. var. PKM 1 using gas chromatography and mass spectroscopy (GC-MS) analysis

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Abstract

The investigation was carried out to study the impact of various organic amendments on the quality of Moringa (*Moringa oleifera* Lam.) at the Western Farm of HC & RI, Periyakulam in Tamil Nadu from 2023-2024. The study employed a factorial randomised block design with three replications. It included thirty different treatments (organic amendments and their combinations), such as enriched farmyard manure (10 t/ha) (S_1), enriched vermicompost (5 t/ha) (S_2), enriched goat manure (8 t/ha) (S_3), enriched poultry manure (8 t/ha) (S_4), a control (without soil application) (S_5), protein hydrolysate (0.3%) (F_1), seaweed extract (3%) (F_2), fulvic acid (2%) (F_3), chitosan (750 ppm) (F_4), orthosilicic acid (0.4%) (F_5), and a control (without foliar spray) (F_6). The results showed that the S_2F_2 (enriched vermicompost (5 t/ha) + 3% seaweed extract + package) achieved the highest levels of crude fat (2.54%), crude fibre (14.95%), crude protein (26.94%), and total carbohydrate content (56.42%). Major components identified in *M. oleifera* leaves from the S_2F_2 treatment included Cis-9,12,15-octadecatrienoic acid (19.14%), sulfurous acid (15.78%), 6-aminoindazole (14.32), n-hexadecanoic acid (6.80), triacontane (4.56%), friedelan-3-one (2.43%), tetradecanoic acid (2.28%), cyclopentadecane (2.25%), eicosane (1.86%), pyrrolid-2-one-5-methanol (1.72%), propanenitrile (1.92%), 2-acetoxy-3-pyrazin-2-ylacrylic acid (1.53%), N-methoxy-N-methylacetamide (1.27%), undecanoic acid (1.16%), (+/-)-2-phenethanamine (1.08%) and methyl 11,14,17-eicosatrienoate (1.07%). Thus, the combined application of vermicompost in the soil and seaweed extract as a foliar spray is deemed the most effective organic amendment for enhancing the phytochemical quality of *M. oleifera*.

1. Introduction

Moringa oleifera Lam. is a fast-growing tree belonging to the Moringaceae family and is native to the sub-Himalayan regions of Northern India. Among the 13 identified species of Moringa, *M. oleifera* is widely cultivated in Africa, Asia, and many Middle Eastern countries (Jikah *et al*., 2023). This adaptable tree, known for its exceptional drought tolerance, has also spread to various tropical and subtropical regions. *M. oleifera* provides numerous edible parts, including seeds, leaves, roots, and flowers, all suitable for human and animal consumption. It is a fast-growing tree, either evergreen or deciduous, that can reach a height of 8 to 12 meters with a trunk diameter of up to 1.5 feet. It thrives in severe drought and mild frost conditions, making it widely cultivated around the world, particularly in tropical and subtropical regions. The young seed pods and leaves are commonly used as vegetables. The leaves, in particular, are rich in protein, vitamins, minerals, β-carotene, and antioxidants, and have been traditionally used in both dietary and medicinal applications (Rawat *et al*., 2024).

Additionally, different parts of this valuable tree are known for their medicinal properties. The flowers, leaves, roots, and seeds have been employed in treating various inflammatory, infectious, cardiovascular, gastrointestinal, hematological and hepatorenal conditions (Sandeep *et al*., 2019). In traditional medicine, Moringa is used for its antioxidant, antimicrobial, anti-inflammatory, antipyretic, antiulcer, antidiabetic, antitumor and hypocholesterolemic effects (James *et al*., 2017).

Phytochemical screening plays a crucial role in identifying new sources of therapeutically and industrially valuable compounds with medicinal significance, enabling the optimal and judicious use of natural resources. Gas chromatography-mass spectroscopy (GC-MS) is an advanced analytical technique used to detect compounds in trace amounts within extracts. It is particularly effective in identifying bioactive compounds, including long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino acids and nitro

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compounds. The identification of unknown organic compounds in complex mixtures is achieved through interpretation and matching with reference spectra (Shunmugapriya *et al*., 2017). This study aims to investigate the phytochemicals present in the methanolic leaf extract of *M. oleifera* and to identify and characterize the bioactive compounds using GC-MS analysis.

2. Materials and Methods

2.1 Collection of sample

The Moringa leaves are harvested in Western Farm, Department of Vegetable Science, Horticultural College and Research Institute, Periyakulam. The location of the experiment is situated at 11.1283° N, 76.5998° E and an altitude of 416 metres above mean sea level. The experiment was designed using a factorial randomized block design (FRBD) with three replications, encompassing 30 treatments. Each treatment included fifty plants, spaced at 1.5 × 0.25 × 0.25 m. The treatments involved five levels of soil application of organic amendments: enriched farmyard manure (10 t/ha) (S_1), enriched vermicompost (5 t/ha) (S_2), enriched goat manure (8 t/ha) (S_3), enriched poultry manure (8 t/ha) (S_4) and control (without soil application) (S_5). Additionally, six levels of foliar application of organic inputs were tested: protein hydrolysate (0.3 %) (F_1), seaweed extract (3 %) (F_2), fulvic acid (2 %) (F_3), chitosan (750 ppm) (F_4), orthosilicic acid (0.4 %) (F_5) and control (without foliar spray) (F_6).

2.2 Proximate composition

The proximate composition of Moringa leaves including moisture, ash, crude fibre, crude fat, crude protein and total carbohydrates was evaluated in triplicate using methods prescribed by the Association of Official Analytical Chemists (AOAC). Ash and moisture contents were determined following AOAC (1995) procedures, while crude fibre was measured using the Maynard method (1970). Nitrogen content was assessed through the Micro-Kjeldahl method (AOAC, 1990), and crude protein concentration was calculated by multiplying the nitrogen percentage by 6.25. Total carbohydrate content was determined by difference using the formula:

Total carbohydrate content (%) = [100 – (Moisture (%) + Ash (%) + Crude Fat (%) + Crude Fiber (%) + Crude Protein (%)].

2.3 Extract preparation of sample

Fresh Moringa leaves were harvested and washed with tap water to remove any remaining debris. They were dried in a shaded area for three to five days, depending on their moisture content. Once dried, the leaves were finely pulverized using a blender. The resulting powder was placed in conical flasks and extracted directly with methanol at a weight/volume ratio of 1:8. This mixture was agitated at 155 rpm for 24 h on an orbital shaker. After the extraction, the mixture was filtered through Whatman filter paper No. 40 (120 mm) to separate the solvent layer. The filtrate obtained was ready for further analysis.

Figure 1: Moringa leaf powder.

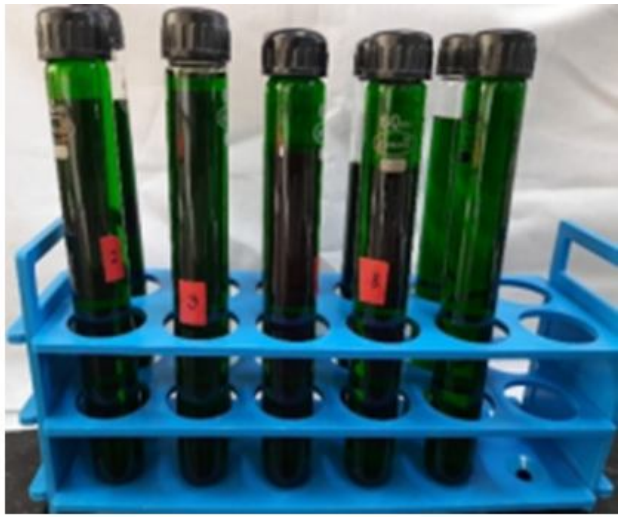


Figure 2: GC-MS sample.

2.4 Gas-chromatography mass-spectrometry (GC-MS) analysis

The required quantity of Moringa leaf powder is placed into a sealed flask, methanol was added to start the extraction process. The mixture was allowed to infuse for 24 h, then filtered and dried using a vacuum distillation apparatus. The resulting residue was subsequently analysed using a GC-MS system. The analysis was conducted with a Thermo GC Ultra Clarus 550 system, which combines a gas chromatograph with a mass spectrometer (GC-MS) equipped with an elite-I fused RMS 6 silica capillary column made of dimethylpolysiloxane. Detection was performed with an electron ionization device set to an ionizing energy of 60 eV. A 1 µl sample was injected with a split ratio of 12:1, and helium (99.9 %) served as the carrier gas at a constant flow rate of 2 ml/min. The ion source and injector temperatures were set to 230°C and 240°C, respectively. The oven temperature was programmed to start at 90°C, increase by 5°C per minute until it reached 240°C, and then remain isothermal for three minutes. Mass spectra were recorded for fragments ranging from 50 to 650 Da, with a scan interval of 0.5 sec. Turbo mass software was used to analyze the mass spectra and chromatograms, and the percentage composition of each component was calculated by comparing the average peak area of each to the total peak area (Dutta *et al*., 2020).

2.5 Identification of bioactive components

The mass spectra from the GC-MS were analyzed and interpreted using the National Institute of Standards and Technology (NIST) database, which includes retention values for over 95,000 compounds (https://www.nist.gov/srd/nist-standard-reference-database1a-v14). Spectra from both the NIST and Wiley libraries were used to match unknown components with known substances. This approach enabled the identification, molecular weight, and compositional analysis of the test materials.

2.6 Biological activity of identified substances

We generated predictions regarding the biological impacts of the compounds using PASS (Prediction of Activity Spectra for Biologically Active Substances), based on their structural formulas. According to the PASS online database (Filimonov *et al*., 2014; Kamaljeet *et al*., 2024), this process involved forecasting a range of pharmacological effects, potential toxicities and possible modes of action associated with the compounds.

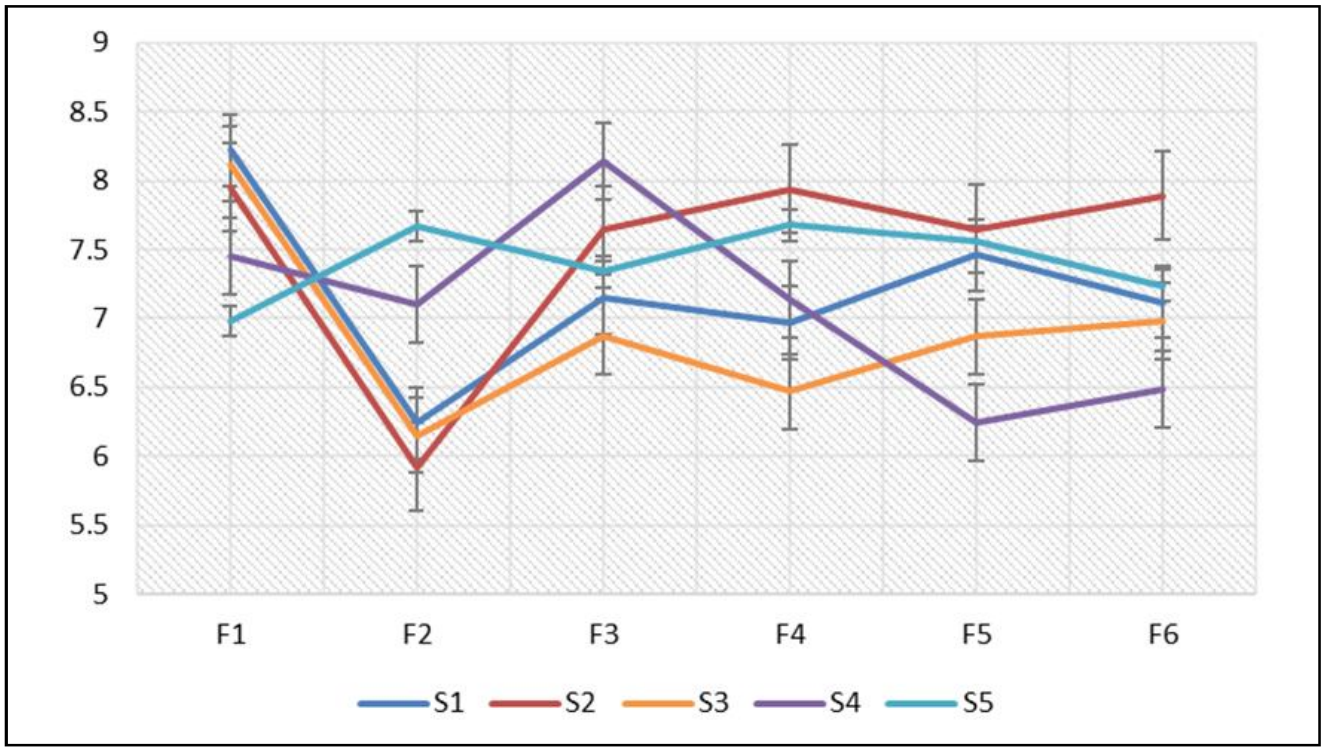


Figure 3: Effect of different organic treatments on moisture content (%).

3. Results

3.1 Proximate analysis

3.1.1 Moisture content (%)

The mean values of moisture content are presented in figure 3. Soil application, foliar application and their interactions significantly influenced the moisture content of Moringa leaves. The moisture content is ranged from 6.94 % to 7.50 %. In soil application, S_4 (enriched poultry manure (8 t/ha)) recorded the lowest moisture content of 6.94 %, followed by S_3 (7.07 %) and the highest moisture content of 7.50 % was recorded in S_2 (enriched vermicompost (5 t/ha)). Among the different foliar spray treatments, the lowest moisture content (6.62 %) was observed in F_2 (seaweed extract (3 %)) followed by F_6 (control) with the moisture content of 7.14 % whereas the highest moisture content (7.74 %) was observed in F_1 (protein hydrolysate (0.3 %)). Among the interaction effect of foliar spray and soil application, S_2F_2 (enriched vermicompost (5 t/ha) + seaweed extract (3 %) + package) recorded the lowest moisture content (5.92 %) followed by S_3F_2 (enriched goat manure (8 t/ha) + seaweed extract (3 %) + package) with the moisture content of 6.15 % and the highest moisture content (8.22 %) was recorded in S_1F_1 (enriched farmyard manure (10 t/ha) + protein hydrolysate (0.3 %) + package).

3.1.2 Ash content (%)

The data pertaining to the ash content showed significant differences among the treatments and the mean values are presented in figure 4. The mean value of ash content is ranged between 6.91 % and 7.41 %. In terms of soil application, S_2 (enriched vermicompost (5 t/ha)) recorded the highest ash content (7.41 %), followed by S4 (7.26 %), while the lowest ash content (6.91 %) was recorded in S_3 (enriched FYM (10 t/ha)). Among the various foliar spray treatments, the highest ash content (8.05 %) was observed in F_2 (seaweed extract (3 %)), followed by F_5 (orthosilicic acid (0.4 %)) with 7.45 %. Conversely, the lowest ash content (6.28 %) was noted in F_1 (protein hydrolysate (0.3 %)). Regarding the interaction effect of foliar spray and soil application, S_2F_2 (enriched vermicompost (5 t/ha) + seaweed extract (3 %) + package) registered the highest ash content (8.72 %), followed by S_1F_2 (enriched farmyard manure (10 t/ha) + seaweed extract (3 %) + package) with the ash content of 8.14 %. In contrast, the lowest ash content (5.24 %) was recorded in S_1F_1 (enriched farmyard manure (10 t/ha) + protein hydrolysate (0.3 %) + package).

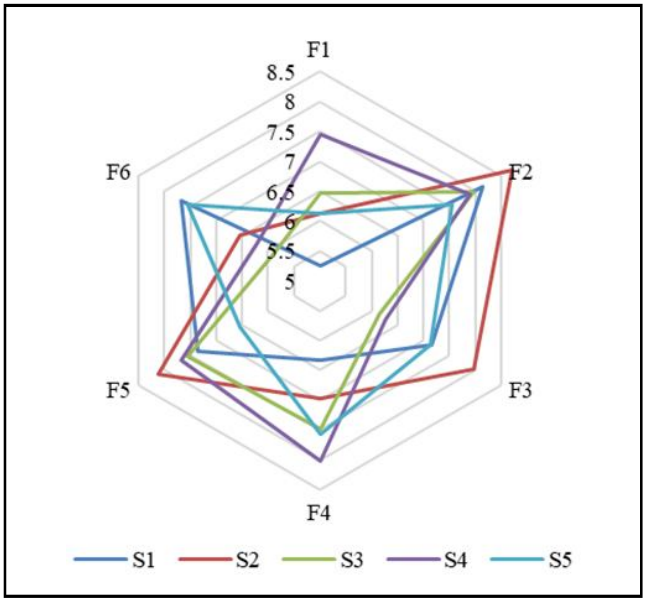


Figure 4: Effect of different organic treatments on ash content (%).

3.1.3 Crude fat content (%)

Observations on the crude fat content are presented in table 1. The mean value of crude fat content varied between 1.50 to 1.83 % among different treatments. In terms of soil application, S_1 (enriched farmyard manure (10 t/ha)) noted the highest crude fat content (1.83 %), followed by S_2 (enriched vermicompost (5 t/ha)) at 1.72 %, while the lowest crude fat content was recorded in S_5 (control) with the value of 1.50 %. Among the foliar spray treatments, F_2 (seaweed extract (3 %)) had the highest crude fat content (2.05 %), followed by F_3 (fulvic acid (2 %)) with 1.78 % of crude fat content. Conversely, the lowest crude fat content (1.26 %) was observed in F_1 (0.3% protein hydrolysate). When considering the interaction between foliar spray and soil application, S_2F_2 (enriched vermicompost (5 t/ha) + 3 % seaweed extract + package) showed the highest crude fat content (2.54 %), followed by S_1F_5 (enriched farmyard manure (10 t/ha) + orthosilicic acid (0.4 %) + package) with 2.39 % of crude fat content. In contrast, the lowest crude fat content (0.97 %) was recorded in S_1F_1 (10 t/ha of enriched farmyard manure + 0.3 % protein hydrolysate + package).

3.1.4 Crude fibre content (%)

The data pertaining to the changes in crude fibre content in Moringa leaves are presented in table 1, with mean values ranging from 13.02 per cent to 14 per cent. Among the soil application treatments, S_5 (control) exhibited the highest crude fibre content of 14.00 per cent, followed by S_2 (enriched vermicompost (5 t/ha)) with 13.92 per cent. Alternatively, S_3 (enriched goat manure (8 t/ha)) displayed the lowest crude fibre concentration of 13.36 per cent. Among the foliar spray treatments, F_5 (orthosilicic acid (0.4 %)) recorded the highest crude fibre content at 13.94 per cent, followed by F_2 (seaweed extract (3 %)) at 13.91 %. Conversely, F_1 (protein hydrolysate (0.3 %)) exhibited the lowest crude fibre concentration at 13.02 per cent. Analyzing the interaction between soil and foliar applications, S_2F_2 (enriched vermicompost (5 t/ha) + seaweed extract (3 %) + package) showcased the highest crude fibre content at 14.95 per cent, while S_2F_5 (Enriched Vermicompost (5 t/ha) + orthosilicic acid (0.4 %) + package) followed closely at 14.91 per cent. On the other hand, the lowest crude fibre content of 12.47 per cent was noted in S_1F_1 (enriched farmyard manure (10 t/ha) + protein hydrolysate (0.3 %) + package).

Table 1: Effect of different organic treatments on crude fat (%) and crude fibre (%)

Treatment	Crude fat (%)						Crude fibre (%)					
	S_1	S_2	S_3	S_4	S_5	Mean	S_1	S_2	S_3	S_4	S_5	Mean
F_1	0.97	1.05	1.45	1.26	1.58	**1.26**	12.47	13.01	13.11	12.53	13.99	**13.02**
F_2	2.14	2.54	1.98	1.85	1.75	**2.05**	13.47	14.95	13.01	13.49	14.65	**13.91**
F_3	2.32	1.97	1.57	1.64	1.38	**1.78**	12.51	12.58	13.08	14.77	13.10	**13.21**
F_4	1.78	1.34	1.79	1.37	1.43	**1.54**	13.54	14.69	13.05	13.80	14.25	**13.87**
F_5	2.39	1.54	1.67	1.78	1.28	**1.73**	13.89	14.91	13.77	12.57	14.55	**13.94**
F_6	1.37	1.89	0.99	1.63	1.57	**1.49**	14.27	13.39	12.99	12.76	13.47	**13.38**
Mean	**1.83**	**1.72**	**1.58**	**1.59**	**1.50**	1.64	**13.36**	**13.92**	**13.17**	**13.32**	**14.00**	**13.55**
	S		**F**		**S x F**		**S**		**F**		**S x F**	
SE (d)	0.07		0.08		0.19		0.03		0.04		0.09	
CD (p = 0.05)	0.15**		0.17*		0.39*		0.07*		0.08*		0.18**	

Note: S_1 - Enriched Farmyard manure (10 t/ha); S_2 - Enriched Vermicompost (5 t/ha); S_3 - Enriched Goat manure (8 t/ha); S_4 - Enriched poultry manure (8 t/ha); S_5 - Control (without Soil application); F_1 - Protein hydrolysate (0.3 %); F_2 - Sea weed extract (3 %); F_3 - Fulvic acid (2 %); F_4 - Chitosan (750 ppm); F_5 - Orthosilicic acid (0.4 %); F_6 - Control (without Foliar spray).

3.1.5 Crude protein content (%)

The observations recorded on the crude protein content of Moringa leaves are depicted in table 2. Within the soil application treatments, S_5 (control) displayed the highest crude protein content at 24.63 per cent, followed by S_4 (enriched poultry manure (8 t/ha)) with 24.52 per cent. Whereas, S_1 (enriched farmyard manure (10 t/ha)) exhibited the lowest crude protein concentration at 22.96 per cent. The highest crude protein content (24.63 %) was observed in F_3 (fulvic acid (2

%)), followed by F_2 (seaweed extract (3 %)) at 24.56 per cent among the foliar treatments. Conversely, F_4 (chitosan (750 ppm)) displayed the lowest crude protein concentration at 23.62 per cent. When considering the interaction between soil and foliar applications, S_3F_2 (enriched goat manure (8 t/ha) + seaweed extract (3 %) + package) showed the highest crude protein content at 26.94 per cent, followed by S_5F_2 control (without soil application) + seaweed extract (3 %) + package) with 26.45 per cent. Whereas, the lowest crude protein content (21.48 %) was observed in S_1F_2 (enriched farmyard manure (10 t/ha) + seaweed extract (3 %) + package).

3.1.6 Carbohydrate content (%)

The observations regarding carbohydrate content are presented in Table 2. The mean values of carbohydrate content ranged from 47.36 % to 49.94 %. Among the soil applications, S_2 (enriched vermicompost (5 t/ha)) recorded the highest carbohydrate content of 49.94 %, which was followed by S_5 (control (without soil application)) with 48.54 % of carbohydrate content. The lowest carbohydrate content of 47.36 % was observed in S_4 (enriched poultry manure (8 t/ha)). Among the foliar spray treatments, the highest carbohydrate content (54.16 %) was observed in F_2 (seaweed extract (3 %)), followed by F_3 (fulvic acid (2 %)) with a crude fibre content of 49.16 % and the lowest carbohydrate content (46.49 %) was observed in F_1 (0.3 % protein hydrolysate). Among the interaction effect of foliar spray and soil application, S_2F_2 (enriched vermicompost (5 t/ha) + seaweed extract (3 %) + package) recorded the highest carbohydrate content (56.42 %) followed by S_4F_2 (enriched poultry manure (8 t/ha) + seaweed extract (3 %) + package) with the carbohydrate content of 55.78 % and the lowest carbohydrate content (42.48 %) was recorded in S_4F_4 (enriched poultry manure (15 t/ha) + chitosan (750 ppm) + package).

Table 2: Effect of different organic treatments on crude protein (%) and carbohydrate content (%)

Treatment	Crude protein (%)						Carbohydrate content (%)					
	S_1	S_2	S_3	S_4	S_5	Mean	S_1	S_2	S_3	S_4	S_5	Mean
F_1	22.43	24.55	22.33	23.11	25.66	23.62	47.21	45.25	46.57	42.78	50.64	46.49
F_2	21.48	22.33	26.94	25.59	26.45	24.56	54.17	56.42	52.78	55.78	51.67	54.16
F_3	26.41	21.58	23.34	25.68	26.12	24.63	49.64	49.47	50.48	46.49	49.74	49.16
F_4	23.13	24.14	25.56	21.52	21.58	23.19	43.65	49.57	46.54	42.48	50.14	46.48
F_5	22.53	24.44	23.33	25.51	21.65	23.49	50.78	47.65	44.65	49.64	41.64	46.87
F_6	21.78	24.46	23.42	25.69	26.30	24.33	48.64	51.26	47.89	46.98	47.39	48.43
Mean	22.96	23.58	24.15	24.52	24.63	23.97	49.02	49.94	48.15	47.36	48.54	48.60
	S		F		S x F		S		F		S x F	
SE (d)	0.08		0.09		0.21		0.81		0.89		2.01	
CD (p=0.05)	0.17*		0.19**		0.43*		1.64**		1.79**		4.01*	

Note: S_1 - Enriched Farmyard manure (10 t/ha); S_2 - Enriched Vermicompost (5 t/ha); S_3 - Enriched Goat manure (8 t/ha); S_4 - Enriched poultry manure (8 t/ha); S_5 - Control (without Soil application); F_1 - Protein hydrolysate (0.3 %); F_2 - Sea weed extract (3 %); F_3 - Fulvic acid (2 %); F_4 - Chitosan (750 ppm); F_5 - Orthosilicic acid (0.4 %); F_6 - Control (without Foliar spray).

Table 3: Different bioactive compounds present in the leaves of Moringa with their IUPAC name, retention time and peak area

S. No.	Compound name	IUPAC name	Molecular weight (g/mol)	Peak area (%)	Retention time (min)
1.	N-methoxy-N-methyl acetamide	N-methoxy-N-methyl acetamide	103.12	1.27	4.609
2.	4,5-diamino-2-hydroxypyrimidine	5,6-diamino-1H-pyrimidin-2-one	126.12	0.69	5.731
3.	4H-pyran-4-one	pyran-4-one	96.08	0.76	6.542
4.	6-aminoindazole	1H-indazol-6-amine	133.15	14.32	9.586
5.	2-methyl-1,3-oxathiolane-2-acetic acid ethyl ester	ethyl 2-(2-methyl-1,3-oxathiolan-2-yl) acetate	190.26	0.80	9.797
6.	2-acetoxy-3-pyrazin-2-ylacrylic acid	ethyl (Z)-2-acetyloxy-3-pyrazin-2-ylprop-2-enoate	236.22	1.53	10.053
7.	1-amino-2-methylnaphthalene	2-methylnaphthalen-1-amine	157.21	0.81	10.542
8.	N-hydroxy-N-ethylcarbamic acid	2-(propoxycarbonylamino)ethyl N-ethyl-N-hydroxycarbamate	234.25	0.90	10.664

9.	Phenylethane	ethynylbenzene	102.13	0.83	10.719
10.	Tetra decanoic acid	tetra decanoic acid	228.37	2.28	10.931
11.	2-methyl-5(6)-cyan benzimidazole	2-methyl-3H-benzimidazole-5-carbonitrile	157.17	0.93	10.986
12.	1-methyl-4-phenyl-1,2,3,6 -tetrahydropyridine	1-methyl-4-phenyl-3,6-dihydro-2H-pyridine	173.25	0.93	11.497
13.	(+/-)-2-phenethanamine	2-methoxy-4-[(1-phenylpropan-2-ylamino) methyl] phenol	271.35	1.08	11.730
14.	Undecanoic acid	Undecanoic acid	186.29	1.16	11.797
15.	5-ethylcyclopent-1-ene-1-carboxy-lic acid	5-ethylcyclopentene-1-carboxylic acid	140.18	0.65	12.041
16.	2,6,6-trimethylbicyclo [3.1.1] heptane	2,6,6-trimethylbicyclo [3.1.1] heptane	138.25	0.69	12.397
17.	n-hexadecenoic acid	hexadecenoic acid	256.42	6.80	13.208
18.	Isophytol	3,7,11,15-tetramethylhexadec-1-en-3-ol	296.50	0.99	14.208
19.	Cis-9,12,15-octadecatrienoic acid	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic	278.40	19.14	14.375
20.	L-arginine	acid (2S)-2-amino-5-(diaminomethy-lideneamino) pentanoic acid	174.20	0.73	15.708
21.	Sulphurous acid	sulphurous acid	82.08	15.78	16.419
22.	Pyrrolid-2-one-5-methanol	5-(hydroxymethyl) pyrrolidin-2-one	115.13	1.72	16.508
23.	Eicosane	Icosane	282.50	1.86	17.463
24.	Methyl 11,14,17-eicosatrienoate	methyl (11E,14E,17E)-icosa-11,14,17 -trienoate	320.50	1.07	17.507
25.	Triacontane	triacontane	422.80	4.56	18.696
26.	Friedelan-3-one	(4R,4aS,6aS,6aS,6bR,8aR,12aR,14aS,14 bS)-4,4a,6a,6b,8a,11,11,14a-octamethyl 2,4,5,6,6a,7,8,9,10,12,12a,13,14, 14b-tetradeca-hydro-1H-picen-3-one	426.70	2.43	19.041
27.	Propane nitrile	propane nitrile	55.80	1.62	20.729
28.	Cyclopentadecanone	cyclopentadecanone	210.40	2.25	22.785
29.	Stigmasterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2 R,5S)-5-ethyl-6-methylhept-3-en-2-yl] -10,13 -dimethyl-2,3,4,7,8,9,11,12,14, 15,16,17- dodecahydro-1H-cyclopenta[a] phenanthren-3-ol	412.70	0.43	22.296
30.	Ethanethiol	ethanethiol	62.14	0.42	16.241

3.2 Metabolite profiling using GC-MS analysis

GC-MS analysis was conducted to identify the bioactive compounds in Moringa leaves. The chromatogram and mass spectrum (Figure 5) indicate that the Moringa leaves from the S_2F_2 treatment (enriched vermicompost (5 t/ha) + seaweed extract (3 %) + package) contain various components. The identified compounds, along with their retention time (RT), peak area (%), and molecular formula, are presented in Table 3. Notably, totally 98 components found in Moringa leaves from the S_2F_2 treatment, the significant compounds such as cis-9,12,15-octadecatrienoic acid (19.14%), sulfurous acid (15.78%), 6-aminoindazole (14.32), n-hexadecanoic acid (6.80), triacontane (4.56%), friedelan-3-one (2.43%), tetradecanoic acid (2.28%), cyclopentadecane (2.25%), eicosane (1.86%), pyrrolid-2-one-5-methanol (1.72%), propanenitrile (1.92%), 2-acetoxy-3-pyrazin-2-ylacrylic acid (1.53%), N-methoxy-N-methylacetamide (1.27%), undecanoic acid (1.16%), (+/-)-2-phenethanamine (1.08%), methyl 11,14,17-eicosatrienoate (1.07%), isophytol (0.99%), 2-methyl-5(6)-cyanobenzimidazole (0.93%), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (0.93%) and N-hydroxy-N-ethylcarbamic acid (0.90%). Whereas, the compounds such as 4,5-diamino-2-hydroxypyrimidine (0.69%), 2,6,6-trimethylbicyclo [3.1.1] heptane (0.69%) and 5-ethylcyclopent-1-ene-1-carboxylic acid (0.65%) are first time reported in Moringa leaves.

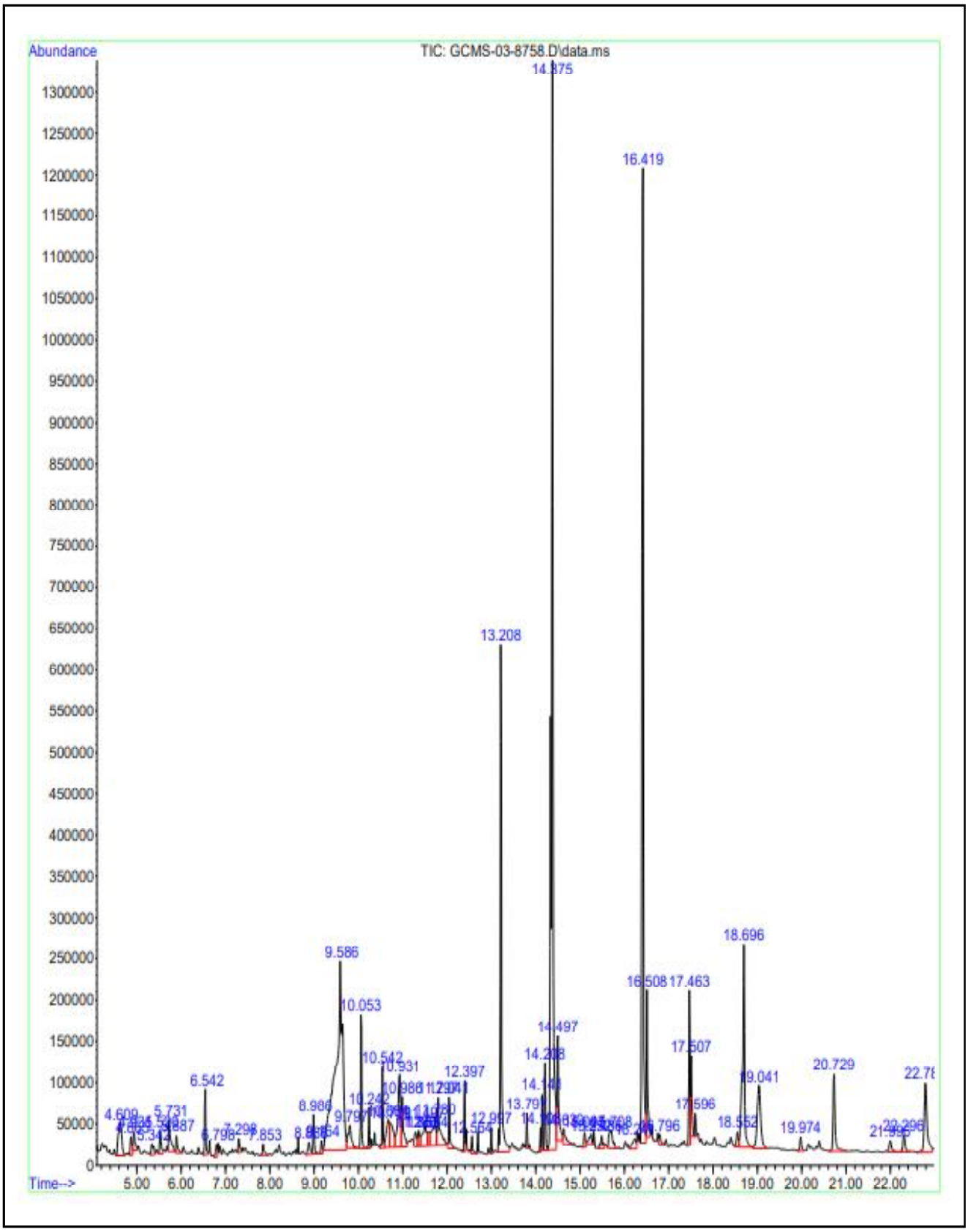
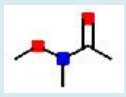
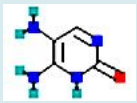
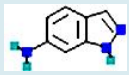
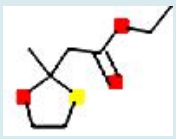
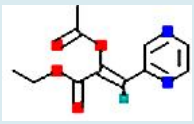
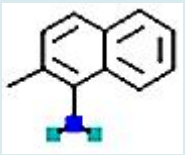
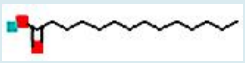
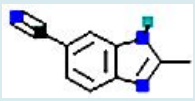
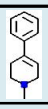
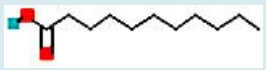
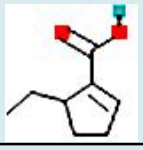
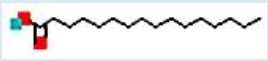
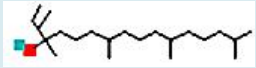
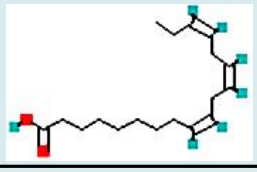
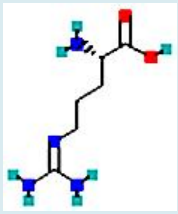
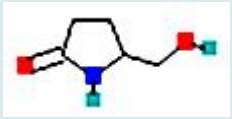
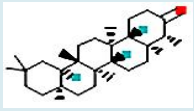
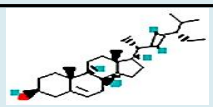
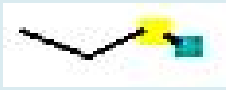


Figure 5: Chromatogram of methanolic extract of Moringa leaves by GC-MS.

Table 4: Moringa leaves components and their pharmaceutical activity with molecular formula and molecular structure identified by GC-MS

S. No.	Compound name	Molecular formula	Molecular structure	Pharmaceutical activity
1.	N-methoxy-N-methyl acetamide	$C_4H_9NO_2$		It is a biochemical reagent that can be utilized as a biological material or organic compound with strong anticancer activity
2.	4,5-Diamino-2-hydrox-	$C_4H_6N_4O$		It is a white, water-soluble solid that is a derivative of ypyrimidine trans-stilbene, featuring amino and sulfonic acid functional groups. This compound is commonly used in cosmetics
3.	4H-Pyran-4-one	$C_5H_4O_2$		It exhibits anti-TB activity and has shown promising antibacterial effects against Gram-positive bacterial strains
4.	6-Aminoindazole	$C_7H_7N_3$		It possesses anti-proliferative activity and is utilized as an antiemetic in cancer chemotherapy.
5.	2-Methyl-1,3-oxathiolane-2-acetic acid ethyl	$C_8H_{14}O_3S$		It has anticancer and antiviral properties and is widely ester used as a solvent, particularly in paints, varnishes, lacquers, cleaning mixtures, and perfumes. It is also used as a solvent for decaffeinating coffee beans.
6.	2-Acetoxy-3-pyrazin-2-ylacrylic acid	$C_{11}H_{12}N_2O_4$		Non-naturally occurring amide compounds that, when added to food, beverages, or pharmaceutical compositions at concentrations around 100 ppm or lower, can enhance savoury flavours.
7.	1-Amino-2-methylnaphthalene	$C_{11}H_{11}N$		Methylnaphthalene is used in the production of certain pesticides or as an additive in some pesticide formulations. 2-Methylnaphthalene is released into the environment during the combustion of wood or fossil fuels or through spills of products containing fossil fuels.
8.	N-Hydroxy-N-ethylcarbamic acid	$C_9H_{18}N_2O_5$		It is the most commonly used insecticide because of its low toxicity to mammals and its short environmental half-life.
9.	Phenylethane	C_8H_6		Treat acute hyperammonemia, which is associated with depression and other mental health disorders.
10.	Tetra decanoic acid	$C_{14}H_{28}O_2$		With its strong antioxidant and radical-scavenging properties, tetradecanoic acid has been assessed for its effectiveness as a penetration enhancer in melatonin transdermal patches in rats and bupropion formulations on human cadaver skin.
11.	2-Methyl-5(6)-cyan benzimidazole	$C_9H_7N_3$		Potential antitumor activity is observed in many commonly used classes of chemotherapeutic agents in medicinal chemistry, which are still molecules that interact with DNA or RNA.
12.	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine	$C_{12}H_{15}N$		A potent toxin used to selectively target and destroy dopaminergic neurons in the *Substantia nigra*, thereby inducing parkinsonism.
13.	(+/-)-2-Phenethanamine	$C_{17}H_{21}NO_2$		With its antileukemic activity, it may also help prevent mood disorders when taken as a supplement.

14.	Undecanoic acid	$C_{11}H_{22}O_2$		A novel therapeutic for fungal infections used as an antifungal agent to treat conditions such as ringworm and athlete''s foot.
15.	5-Ethylcyclopent-1-ene-1 -carboxylic acid	$C_8H_{12}O_2$		They assist in maintaining the cell membrane and regulating nutrient usage and metabolism.
16.	2,6,6-trimethylbicyclo [3.1.1] heptane	$C_{10}H_{18}$		It has been studied for its pharmacological effects, especially its ability to inhibit the uptake of neuronal 5-hydroxytryptamine (5-HT).
17.	n-Hexadecenoic acid	$C_{16}H3_2O_2$		It has been shown to regulate inflammation by inhibiting phospholipase A2, indicating its potential as an anti-inflammatory agent. Additionally, n-hexadecanoic acid is associated with neurotrophic effects, making it a promising candidate for both prophylactic and therapeutic use in neuropathy.
18.	Isophytol	$C_{20}H_{40}O$		It is frequently used as a precursor in the production of synthetic forms of vitamin E and vitamin K_1.
19.	Cis-9,12,15-Octadecatrie-noic acid	$C_{18}H_{30}O_2$		Bioactive natural products, *In vivo* production of CLNA as a tool for regulating host microbiota in obesity control and the development and modification of bioactivity.
20.	L-Arginine	$C_6H_{14}N_4O_2$		Used to lower blood pressure and support protein synthesis. L-arginine is typically produced in sufficient amounts by the body. It is also present in various protein-rich foods, such as fish, red meat, poultry, soy, whole grains, beans, and dairy products. As a supplement, L-arginine can be applied both orally and topically.
21.	Sulphurous acid	H_2O_3S		An antimicrobial agent that inhibits bacteria, yeasts, and molds, and is used both as a reducing agent and a disinfectant.
22.	Pyrrolid-2-one-5-methanol	$C_5H_9NO_2$		Anticholinergic effects are utilized to manage symptoms of allergic rhinitis. Additionally, a lincosamide antibiotic is used to treat severe infections caused by susceptible anaerobic, streptococcal, staphylococcal, and pneumococcal bacteria.
23.	Eicosane	$C_{20}H_{42}$		It has very potent anti-inflammatory, analgesic and antipyretic effects.
24.	Methyl 11,14,17-eicosatri enoate	$C_{21}H_{36}O_2$		Its potent anti-thrombogenic, anti-inflammatory and anti-atherogenic properties
25.	Triacontane	$C_{30}H_{62}$		Cocarcinogenic activity
26.	Friedelan-3-one	$C_{30}H_{50}O$		Antioxidant, antimicrobial, antipyretic, antiulcer, anticonvulsant, and antitumor activities

27.	Propane nitrile	C_3H_5N		It is a simple aliphatic nitrile, existing as a colourless, water-soluble liquid. This compound is used as a solvent and as a precursor to other organic compounds.
28.	Cyclopentadecanone	$C_{15}H_{30}$		In the treatment of ischemic cerebrovascular disease, this drug also addresses antirheumatic and rheumatoid conditions, as well as helps prevent tumours.
29.	Stigmasterol	$C_{29}H_{48}O$		It has antidiabetic effects by lowering fasting glucose levels and serum insulin levels and improving oral glucose tolerance.
30.	Ethanethiol	C_2H_6S		It is added to odourless gaseous products like liquefied petroleum gas (LPG) to impart a garlic scent, which helps detect gas leaks. Additionally, it serves as a rodenticide.

4. Discussion

M. oleifera is widely acknowledged as a major source of organic antioxidants worldwide. Plants contain phytochemicals that can be used to make medicines because they have unique physiological effects on humans (Goel *et al*., 2022). The study's findings indicate that the moisture content of Moringa leaves is within the predicted range. Leaves with high moisture content are highly perishable and prone to microbial deterioration when stored. Because of its comparatively low moisture content, Moringa has a longer shelf-life and inhibits the growth of microorganisms (Thapa *et al*., 2019; Jyoti *et al*., 2024).

The ash value indicates that Moringa plants are a good source of inorganic minerals. A high mineral deposit is indicated by a high ash content in food (Peñalver *et al*., 2022; Abdulbaseer *et al*., 2024). Comparing the fat content of this study to that of other plants, it is moderate. Through the absorption and retention of flavours, dietary fats contribute to the increased palatability of food (Suman *et al*., 2022; Anuradha *et al*., 2024). Humans are considered to require a diet that contains no more than 2 % of their energy in the form of fat, as consuming too much fat has been linked to various cardiovascular conditions like atherosclerosis, cancer and ageing (Bolarinwa *et al*., 2019). Despite being low in nutrients, consuming enough crude fiber can help lower blood cholesterol, improve the absorption of trace minerals and other nutrients in the gut, and reduce the risk of heart disease, diabetes, colon and breast cancer, constipation, and hypertension. (Aggarwal *et al*., 2022). According to diet, the study's findings on the fibre content of Moringa are consistent with earlier research and adequate. According to the protein value of our sample, Moringa contains high-quality proteins that are suitable for human and animal diets and can effectively meet daily protein requirements (Patil *et al*., 2022; Amad *et al*., 2022). The natural synthesis and maintenance of bodily tissues, hormones, enzymes, and other chemicals necessary for proper operation depend on dietary proteins. A plant-based food is considered a good source of protein if more than 12% of its total caloric content comes from protein (Chen *et al*., 2020; Islam *et al*., 2020 and Shenbagavalli *et al*., 2024). Moringa has a high carbohydrate content compared to other crops, which implies that it could be a effective supplement as a source of energy and organic materials for muscle building. Typically making up the majority of a diet, carbohydrates are essential because they give blood, muscles, and brain cells energy. In addition to serving as a mild laxative for people, they aid in the metabolism of fat and spare proteins for energy (Chigurupati *et al*., 2022).

Among the various organic amendments, the S_2F_2 treatment (enriched vermicompost (5 t/ha) + seaweed extract (3 %) + package) yielded the highest production and richest phytochemical composition. Therefore, the best-performing treatments were collected for GC-MS analysis. The GC-MS chromatogram (Figure 5), illustrates the diverse phytochemical compounds found in Moringa leaves under the S_2F_2 treatment and Table 4 provides a comprehensive list of these bioactive compounds, detailing their molecular weights and pharmacological activities.

5. Conclusion

The study concludes that the quality attributes of *M. oleifera* were significantly improved through the combined use of two organic nutrient sources: vermicompost and seaweed extract. Among the various treatments, S_2F_2 (enriched vermicompost (5 t/ha) + seaweed extract (3 %) + package) and a set of supplementary treatments produced the most favourable results across all quality parameters for Moringa. The GC-MS analysis further supports the notion that Moringa may have significant therapeutic and pharmacological benefits for humans. The methanolic extract of Moringa leaves identified over 98 compounds, providing scientific evidence for their pharmacological potential. The compounds detected under GC-MS exhibited a wide range of biological activities, including hepatoprotective, antioxidant, antimicrobial, antifungal, anti-inflammatory, and antitumor effects, making them highly valuable for pharmacological industries. This analysis highlights the diverse active compounds present in Moringa leaves and underscores its significance. As a result, pharmaceutical researchers and industries are increasingly relying on Moringa as a crucial raw material for drug development and production.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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