

Phytochemical Characterization of Aqueous Extract of *Moringa oleifera* Seeds Using Gas Chromatography – Mass Spectrometry (GC-MS) and Gas Chromatography – Flame Ionization Detection (GC-FID)Onyewuchi G. Ugwuibe¹, Emmanuel N. Agomuo², Raphael C. Ekeanyanwu², Adaeze B. Chile-Agada², Lien-Mary Nkemjika-Aguwa²¹ Department of Biochemistry, Faculty of Science, Kingsley Ozumba Mbadie University (KOMU), Ideato, Imo State, Nigeria.² Department of Biochemistry, Faculty of Biological Sciences, Imo State University, Owerri, Imo State, Nigeria.**ABSTRACT**

Moringa oleifera seeds constitute an important reservoir of bioactive phytochemicals with established nutritional and pharmacological significance. The present study characterized the phytochemical composition of the aqueous extract of *Moringa oleifera* seeds using gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame ionization detection (GC-FID). Extraction was performed using controlled aqueous maceration, followed by chromatographic analysis under optimized instrumental conditions. GC-MS profiling revealed thirty-five putatively identified constituents belonging to diverse phytochemical classes, including phenylpropanoids, flavonoids, phenolic derivatives, fatty acids, esters, alkanes, alkenes, and related aromatic compounds. Constituents detected included cinnamic acid, sinapic acid, epicatechin, 2,4-di-tert-butylphenol, erucic acid, palmitoleic acid, and several long-chain fatty acid derivatives. GC-FID analysis further revealed the presence of biologically important flavonoids and phenolic compounds such as catechin, apigenin, quercetin, myricetin, genistein, resveratrol, luteolin, kaempferol, and naringenin. The predominance of phenolic acids and flavonoids within the extract suggests substantial antioxidant and redox-modulating potential. Structural characteristics of the identified compounds, particularly hydroxylated phenolic and flavonoid moieties, indicate possible involvement in free radical scavenging, modulation of oxidative stress, and regulation of inflammatory signaling pathways. The combined GC-MS and GC-FID analyses demonstrate that the aqueous seed extract of *Moringa oleifera* possesses considerable phytochemical diversity with potential biological and pharmacological relevance. These findings provide a chemical basis for the traditional applications of *Moringa oleifera* seeds and support further pharmacological, toxicological, and bioactivity-guided investigations.

Keywords: *Moringa oleifera*; gas chromatography-mass spectrometry, gas chromatography-flame ionization detection; phytochemical profiling, flavonoids, phenolic compounds, bioactive constituents.

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Introduction

Medicinal plants continue to represent an important source of structurally diverse bioactive compounds with considerable nutritional and pharmacological relevance. The biological activities associated with plant-derived extracts are largely attributed to secondary metabolites such as phenolic compounds, flavonoids, alkaloids, terpenoids, and fatty acid derivatives. These phytochemicals have attracted increasing scientific interest because of their reported antioxidant, anti-inflammatory, antimicrobial, and cytoprotective properties, particularly in conditions associated with oxidative stress and metabolic dysfunction.^{1,2,3,4}

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Phenolic and polyphenolic compounds are especially recognized for their ability to scavenge reactive oxygen species, stabilize free radicals, and regulate redox-sensitive cellular pathways involved in inflammation and oxidative injury.^{5,6,7}

Moringa oleifera Lam. (Family: Moringaceae) is a medicinal plant widely cultivated in tropical and subtropical regions because of its nutritional value and diverse therapeutic applications. Different parts of the plant, including the leaves, seeds, roots, and bark, have traditionally been utilized in the management of inflammatory disorders, microbial infections, metabolic abnormalities, and oxidative stress-related conditions.^{8,9,10} Although substantial scientific attention has focused on the leaves of *M. oleifera*, recent investigations have demonstrated that the seeds also possess a rich phytochemical composition containing phenolic acids, flavonoids, lipid-derived compounds, and other secondary metabolites of potential biological importance.^{8,11,12}

The phytochemical constituents of *M. oleifera* seeds have been associated with several biological activities, including antioxidant, anti-inflammatory, cytoprotective, and neuroprotective effects. Flavonoids and phenolic acids present in medicinal plants are known to exert antioxidant activities through mechanisms involving hydrogen atom donation, metal ion chelation, and modulation of redox-sensitive signaling pathways such as nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor-kappa B (NF-κB).^{5,13,14} In addition, fatty acids and related lipid-derived phytochemicals contribute to membrane integrity, metabolic regulation, and cellular signaling processes.⁹ The structural diversity of these compounds therefore

contributes significantly to the pharmacological and biochemical relevance of *M. oleifera* seed extracts.

Reliable phytochemical characterization of medicinal plant extracts requires sensitive analytical techniques capable of identifying structurally diverse compounds within complex biological matrices.¹⁵ Gas chromatography-mass spectrometry (GC-MS) remains one of the most widely employed analytical approaches for the characterization of volatile and semi-volatile phytochemicals based on chromatographic retention patterns and mass spectral fragmentation profiles. Similarly, gas chromatography with flame ionization detection (GC-FID) provides high sensitivity for the detection and relative quantification of organic compounds, particularly fatty acids, hydrocarbons, and phenolic constituents.^{16,17,18} The combined application of GC-MS and GC-FID therefore offers complementary analytical information that enhances the reliability and depth of phytochemical profiling.

Despite increasing scientific interest in *Moringa oleifera*, detailed phytochemical characterization of aqueous seed extracts using combined GC-MS and GC-FID analytical approaches remains limited. This is particularly important because aqueous extracts are commonly utilized in traditional medicine and experimental investigations. Therefore, the present study investigated the phytochemical composition of the aqueous extract of *Moringa oleifera* seeds using GC-MS and GC-FID techniques in order to provide analytical insight into its bioactive constituents and potential biological relevance.

Materials and Methods

Plant Material Collection and Authentication

Moringa oleifera seeds used in this study were obtained from mature seed pods harvested from a cultivated farm located in Area G, New Owerri, Owerri, Imo State, Nigeria, in May 2024. The seeds were manually removed from the pods, air-dried under ambient laboratory conditions, and pulverized into fine powder using a mechanical grinder. The powdered samples were stored in airtight containers under refrigerated conditions at approximately 4 degrees C prior to extraction.

The plant material was taxonomically authenticated as *Moringa oleifera* Lam. (Family: Moringaceae), commonly known as the drumstick tree and locally referred to as Okwe Oyibo in Igbo. Authentication was carried out by Mr. Iwueze Francis Onyemauche of the Department of Forestry and Wildlife Technology, Federal University of Technology, Owerri (FUTO), Nigeria. A voucher specimen was deposited in the departmental herbarium under the reference number FUTO/FWT/ERB/2025/119 for future reference.

Preparation of Aqueous Extract of *Moringa oleifera* Seeds

The aqueous extract of *Moringa oleifera* seeds was prepared using controlled maceration procedures. Briefly, 2500 g of the pulverized seed powder was macerated in 12,500 mL of distilled water at 80 degrees C with continuous agitation at 100 rpm for 48 h. The resulting mixture was filtered using Whatman No. 1 filter paper to remove particulate materials.

The filtrate was concentrated under reduced pressure using a rotary evaporator maintained at 40 degrees C and subsequently freeze-dried to obtain a solid extract. The dried extract was stored under refrigeration at 4 degrees C until further analysis.

Extraction yield was calculated as percentage yield relative to the initial weight of the powdered sample, and the yield obtained was 7.6%. The extraction procedure was performed according to established phytochemical extraction methodologies.^{19,20}

GC-MS Analysis

Gas chromatography-mass spectrometry (GC-MS) was performed using an Agilent GC-MS instrument fitted with an HP-5MS capillary column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). Helium served as the carrier gas and was maintained at a constant flow rate of 1.0 mL/min. A sample volume of 1 µL was injected using a 10:1 split ratio, with the injector temperature maintained at 250 °C.

Chromatographic separation was initiated at an oven temperature of 60 °C, which was held for 2 min. The temperature was subsequently increased at 10 °C/min to 280 °C and maintained at the final temperature for 10 min. Mass spectral detection was performed in electron ionization mode at an ionization energy of 70 eV, with mass spectra acquired over an *m/z* range of 40–600.¹⁶

GC-FID Analysis

Gas chromatography with flame ionization detection (GC-FID) was carried out using an Agilent gas chromatograph equipped with a flame ionization detector and a capillary column comparable to that employed for GC-MS analysis.

Nitrogen was used as the carrier gas at a flow rate of 1.0 mL/min. The injection volume was maintained at 1 µL, while chromatographic separation was performed in split mode using a split ratio of 10:1. The injector and detector temperatures were maintained at 250 degrees C and 300 degrees C, respectively.

The oven temperature program followed conditions similar to those employed for GC-MS analysis, beginning at 60 degrees C and increasing progressively to 280 degrees C at a rate of 10 degrees C/min with a final hold time of 10 min.¹⁷

Identification of Phytochemical Constituents

Phytochemical constituents detected by GC-MS analysis were identified through comparison of the obtained mass spectra with spectra contained in the National Institute of Standards and Technology (NIST) mass spectral database. Compound identification was based on spectral matching, retention characteristics, and fragmentation patterns.

The relative abundance of the identified compounds was determined from chromatographic peak area percentages obtained from the corresponding chromatograms. GC-FID analysis was further employed to support the detection and relative quantification of selected phytochemical constituents based on detector response characteristics.

Compounds with similarity indices greater than or equal to 80% were considered reliably identified, while compounds with lower similarity scores were interpreted cautiously as tentatively identified constituents.

Statistical Analysis

The chromatographic data generated in this study were analyzed descriptively. GC-MS results were expressed in terms of retention time, relative peak area (%) and spectral library-match quality, while GC-FID data were reported as retention time, peak area and concentration (ppm). As the study was designed for qualitative and quantitative phytochemical profiling rather than comparison between experimental groups, no inferential statistical tests were applied.

Results and Discussion

GC-MS Profile of the Aqueous Extract of *Moringa oleifera* Seeds

Gas chromatography-mass spectrometry (GC-MS) profiling of the aqueous extract of *Moringa oleifera* seeds revealed 35 constituents distributed among several chemical classes, including phenylpropanoids, phenolic compounds, fatty acids and their derivatives, esters, alkanes, alkenes, aldehydes, and related aromatic compounds (Table 1). This diverse chemical profile indicates that the aqueous seed extract contains compounds spanning both relatively polar phytochemical groups and lipid-derived constituents.

Among the prominent constituents were cinnamic acid, sinapic acid, and 2,4-di-tert-butylphenol, with peak areas of 9.25%, 9.26%, and 9.67%, respectively. Other constituents included erucic acid, palmitoleic acid, cis-vaccenic acid, fatty acid esters, and several long-chain hydrocarbons. Epicatechin was also recorded among the GC-MS library matches. Collectively, the profile demonstrates considerable chemical diversity in the aqueous seed extract.

The occurrence of cinnamic and sinapic acids is particularly relevant to the phenolic composition of the extract. Phenolic acids, including

hydroxycinnamic acid derivatives, have been reported to exhibit antioxidant and anti-inflammatory activities associated with their capacity to donate hydrogen atoms, stabilize reactive intermediates, chelate metal ions, and influence oxidative and inflammatory pathways^{6,21}. The biological significance attributed to these compounds in previous studies therefore provides a useful context for interpreting their occurrence in the present extract, although biological activity was not directly evaluated in this study.

The detection of 2,4-di-tert-butylphenol further broadens the phenolic profile obtained by GC-MS. Substituted phenolic compounds are known for structural characteristics that permit stabilization of radical intermediates and interruption of oxidative chain reactions. Accordingly, its presence, together with the phenylpropanoid constituents, identifies phenolic compounds as an important component of the chemical profile of the aqueous seed extract.

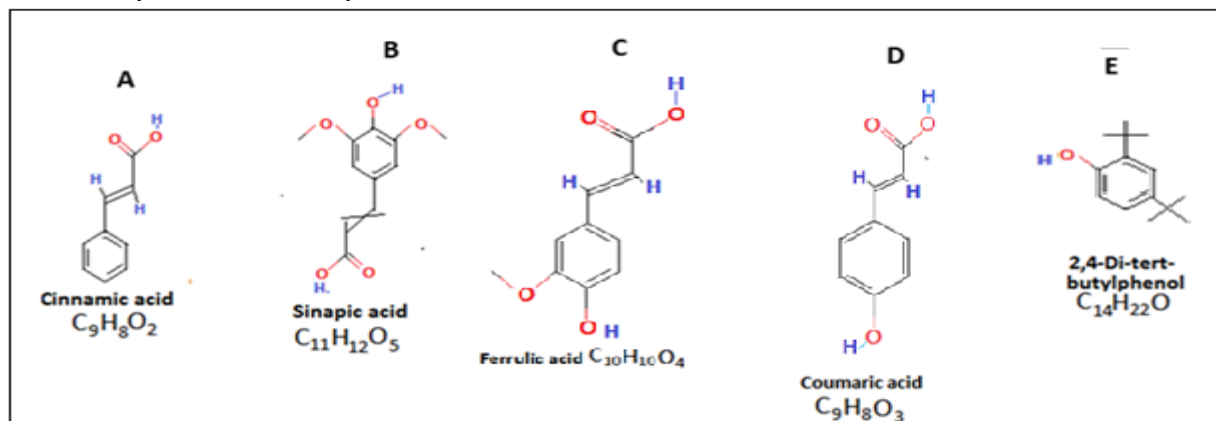


Figure 1: Structures of selected phenylpropanoid and phenolic compounds detected in the aqueous extract of *Moringa oleifera* seeds: (A) cinnamic acid, (B) sinapic acid, (C) ferulic acid, (D) coumaric acid, and (E) 2,4-di-tert-butylphenol.

GC-FID Profile and Distribution of Flavonoid and Phenolic Constituents

Gas chromatography-flame ionization detection (GC-FID) provided an additional profile comprising 25 constituents classified mainly as flavonoids, phenolic acids, stilbenes, terpenoids, and related compounds (Table 2). Prominent constituents based on the reported concentrations included apigenin (49.06588 ppm), limonene (25.50425 ppm), myricetin (15.25054 ppm), quercetin (5.26606 ppm), nobiletin (4.68398 ppm), flavone (4.70277 ppm), epicatechin (3.88271 ppm), resveratrol (3.83066 ppm), genistein (3.12932 ppm), and kaempferol (3.11893 ppm).

A notable feature of the GC-FID profile was the representation of several flavonoid subclasses. Flavones, flavonols, flavanols, flavanones, isoflavones, and their derivatives were represented by compounds such as apigenin, luteolin, quercetin, myricetin, kaempferol, catechin, epicatechin, naringenin, and genistein. Phenolic constituents, including cinnamic, ferulic, coumaric, and vanillic acids, were also recorded. The range of compounds detected therefore indicates considerable phytochemical heterogeneity within the aqueous seed extract.

The structural arrangement of flavonoids, particularly the number and position of hydroxyl groups and the extent of conjugation within their ring systems, is an important determinant of their reported biological properties. Flavonoids have been associated with antioxidant, anti-inflammatory, and cytoprotective effects through mechanisms that include scavenging of reactive oxygen species and modulation of redox-sensitive signaling pathways such as nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor-kappa B (NF-κB).^{1,3,5,14} Catechin and epicatechin are recognized for radical-scavenging activity, while the hydroxylation patterns of quercetin and myricetin contribute to their reported antioxidant properties.^{1,5} Apigenin, luteolin, and naringenin have likewise been associated with modulation of inflammatory signaling and protection against oxidative injury in experimental systems.^{1,5} Genistein and resveratrol have also been investigated for their interactions with cellular signaling, metabolic regulation, and apoptosis. These literature-reported activities should, however, be interpreted as properties associated with the individual compounds rather than direct evidence of such activities in the aqueous seed extract examined in the present study.

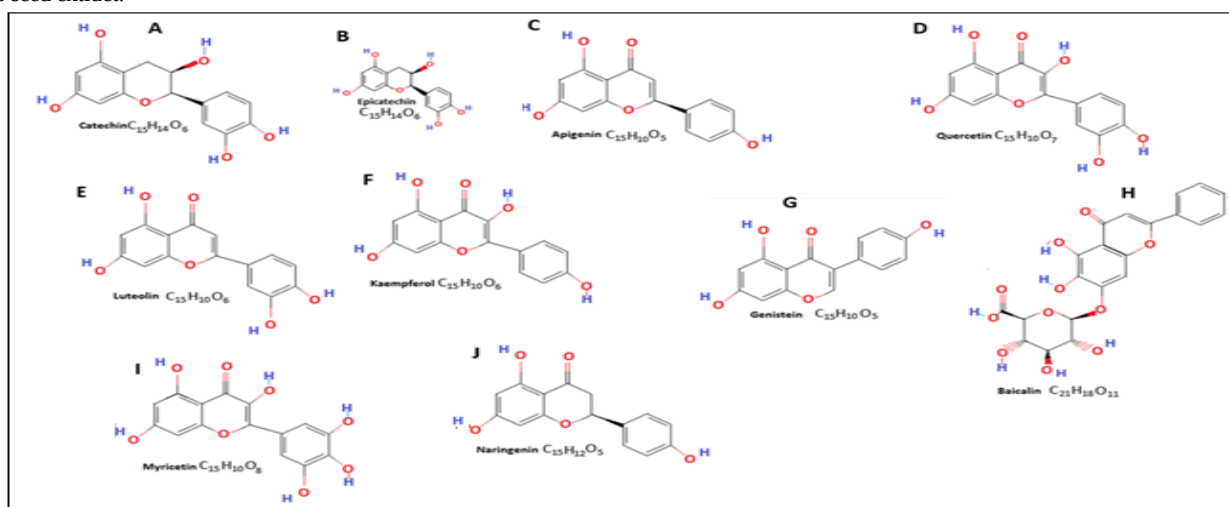


Figure 2: Structures of selected flavonoids detected in the GC-FID profile of aqueous extract of *Moringa oleifera* seeds: (A) catechin, (B) epicatechin, (C) apigenin, (D) quercetin, (E) luteolin, (F) kaempferol, (G) genistein, (H) baicalin, (I) myricetin, and (J) naringenin.

Fatty Acids and Lipid-Derived Constituents

The GC-MS profile also contained several fatty acids and lipid-derived constituents. Erucic acid, palmitoleic acid, cis-vaccenic acid, trans-13-octadecenoic acid, and different octadecenoic acid derivatives were among the compounds recorded (Table 1). Their occurrence adds a lipid-associated component to the otherwise phenolic-rich phytochemical profile of the extract.

Fatty acids are involved in membrane organization, cellular signaling, and metabolic processes, with their physicochemical behavior being influenced substantially by chain length and degree of unsaturation^{8,9}. The occurrence of unsaturated fatty acids in the present analysis is therefore of biochemical interest. Nevertheless, their detection alone does not establish a specific physiological effect of the extract. This distinction is particularly important for erucic acid, for which biological effects are dependent on the extent and duration of exposure.

Table 1: GC-MS putatively identified compounds in aqueous extract of *Moringa oleifera* seeds

S/N	Compound	Chemical formula	Class of phytochemical	RT	Area (%)	Quality
1	Cinnamic acid (CA)	C ₉ H ₈ O ₂	Phenylpropanoid	5.238	9.25	87
2	Hexadecane, 1-chloro- (H1Cl)	C ₁₆ H ₃₃ Cl	Alkyl halide	6.884	1.34	52
3	Benzene, 1,4-dichloro (para-dichlorobenzene)	C ₆ H ₄ Cl ₂	Chlorinated aromatic hydrocarbon	7.426	0.27	96
4	Carbonic acid, decyl tetradecyl ester	C ₂₀ H ₃₄ O ₄	Carbonic acid ester	8.522	1.20	46
5	17-Pentatriacontene	C ₃₅ H ₇₀	Alkene	8.878	3.34	80
6	Undecane, 1-bromo-	C ₁₁ H ₂₄ Br	Haloalkane	8.985	3.25	50
7	Tetracosane	C ₂₄ H ₅₀	Alkane	9.138	1.45	62
8	Sinapic acid	C ₁₀ H ₁₂ O ₄	Phenylpropanoid derivative	9.430	9.26	87
9	Decane, 3,8-dimethyl-	C ₁₀ H ₂₂	Branched alkane	9.558	1.70	53
10	Heptane, 2,6-dimethyl-	C ₉ H ₂₀	Branched alkane	9.621	3.32	72
11	Carbonic acid, nonyl prop-1-en-2-yl ester	C ₂₂ H ₄₀ O ₄	Carbonic acid ester	9.815	8.64	86
12	Epicatechin	C ₁₅ H ₁₄ O ₆	Flavanol	10.159	8.34	64
13	Hydroxylamine, O-decyl-	C ₁₀ H ₂₃ NO	Hydroxylamine derivative	10.579	2.09	49
14	Tetracontane, 3,5,24-trimethyl-	C ₄₃ H ₈₈	Branched alkane	12.804	1.34	72
15	Tridecane	C ₁₃ H ₂₈	Straight-chain alkane	15.708	1.72	76
16	1-Nonadecene	C ₁₉ H ₃₈	Linear alkene	18.284	1.78	92
17	Palmitic acid vinyl ester	C ₁₈ H ₃₄ O ₂	Fatty acid ester	18.478	1.45	89
18	1-Decanol, 2-hexyl-	C ₁₆ H ₃₄ O	Long-chain alcohol	21.107	0.93	72
19	2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	Phenolic compound	22.130	9.67	97
20	Erucic acid	C ₂₂ H ₄₂ O ₂	Monounsaturated fatty acid	23.442	2.34	83
21	1-Octadecene	C ₁₈ H ₃₆	Linear alkene	28.091	2.97	95
22	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	Phthalate ester	30.529	1.45	94
23	3-Eicosene, (E)-	C ₂₀ H ₄₀	Alkene	30.704	2.54	98
24	1-Ethanone, 1-[4-acetyl-2,5-dimethyl-1-(8-quinolinyl)-1H-pyrrol-3-yl]-	C ₈ H ₁₀ NO	Heterocyclic aromatic compound	30.886	0.64	38
25	Palmitoleic acid	C ₁₆ H ₃₀ O ₂	Monounsaturated fatty acid	32.036	0.57	91
26	1-Docosene	C ₂₂ H ₄₄	Alkene	32.181	1.27	97
27	9-Octadecenal, (Z)-	C ₁₈ H ₃₄ O	Aldehyde	32.591	2.05	91
28	2-Piperidinone, N-[4-bromo-n-butyl]	C ₉ H ₁₆ BrNO	Lactam	32.934	3.41	42
29	Cis-13-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	Monounsaturated fatty acid	33.106	2.36	45
30	13-Octadecenal, (Z)-	C ₁₈ H ₃₄ O	Aldehyde	33.225	0.64	56
31	Cis-vaccenic acid	C ₁₈ H ₃₄ O ₂	Monounsaturated fatty acid	33.375	2.11	74
32	9-Octadecenoic acid (Z)-, tetradecyl ester	C ₃₂ H ₆₂ O ₂	Fatty acid ester	33.679	1.61	43
33	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	C ₂₁ H ₄₀ O ₅	Fatty acid ester	33.875	0.97	38
34	7-Tetradecenal, (Z)-	C ₁₄ H ₂₆ O	Unsaturated fatty aldehyde	34.000	0.95	50
35	Trans-13-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	Monounsaturated fatty acid	34.116	1.42	78

RT = retention time.

Fatty acid esters were also represented in the chromatographic profile. Together with the free fatty acids, these compounds demonstrate that

the aqueous seed extract contains structurally diverse lipid-derived constituents in addition to its phenolic and flavonoid components.

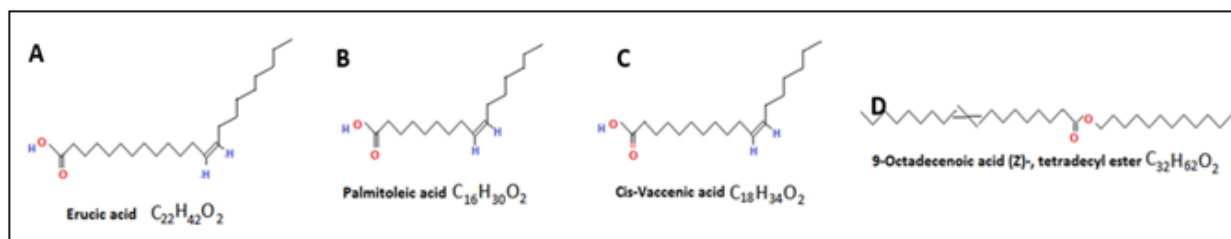


Figure 3: Structures of selected fatty acids and fatty acid esters detected in the GC-MS profile of aqueous extract of *Moringa oleifera* seeds: (A) erucic acid, (B) palmitoleic acid, (C) cis-vaccenic acid, and (D) 9-Octadecenoic acid (Z)- tetradecyl ester

Hydrocarbons, Terpenoids, and Related Constituents

Long-chain hydrocarbons and related compounds constituted another component of the GC-MS profile. These included tetracosane, tridecane, 17-pentatriacontene, 1-nonadecene, 1-octadecene, 1-docosene, and other hydrocarbon derivatives. Limonene, a monoterpene, was additionally recorded in the GC-FID profile. Hydrocarbons occur naturally in plants and may contribute to structural and protective functions, while terpenoids represent a

chemically diverse group with a range of reported biological properties. Limonene, for example, has been investigated for antioxidant and anti-inflammatory activities. Its detection in the present analysis should primarily be regarded as part of the phytochemical fingerprint of the seed extract because the present investigation did not directly assess these biological effects.

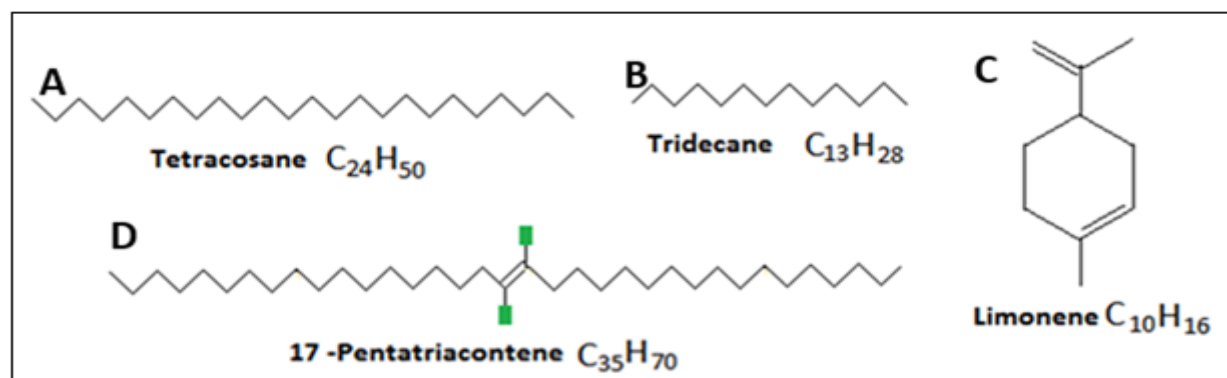


Figure 4: Structures of selected hydrocarbons and terpenoid compounds detected in the chromatographic profiles of aqueous extract of *Moringa oleifera* seeds: (A) tetracosane, (B) tridecane, (C) limonene, and (D) 17-pentatriacontene

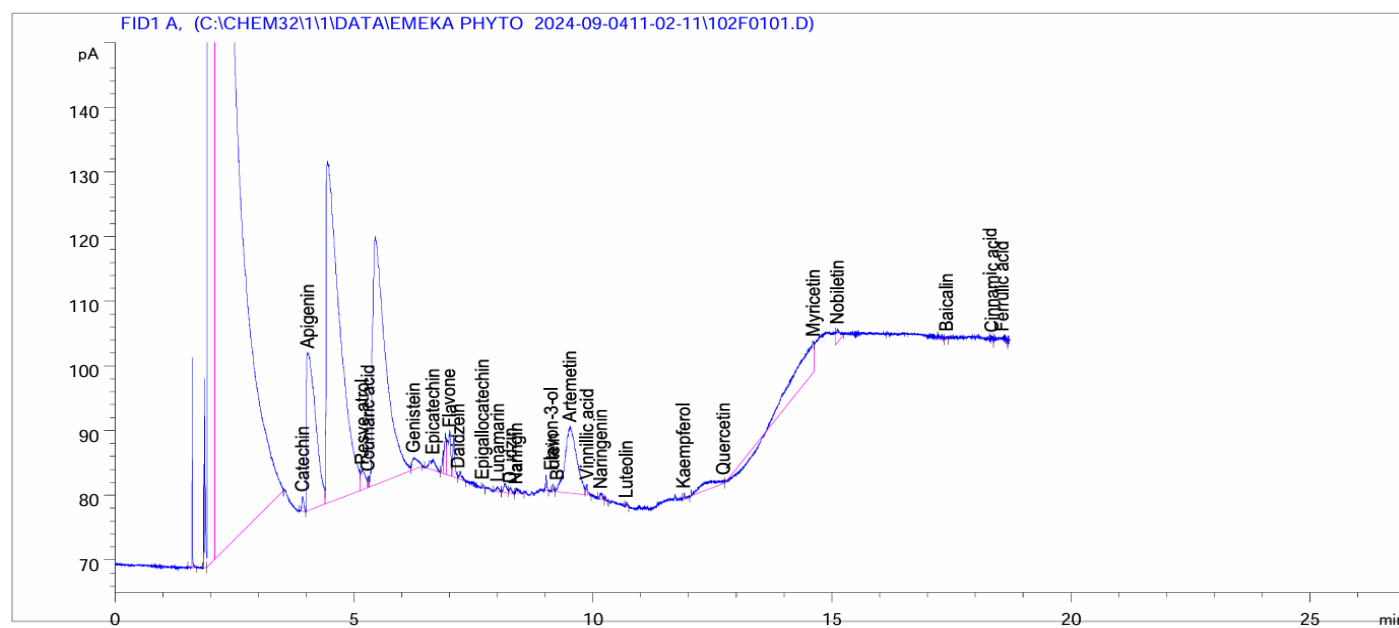


Figure 5: GC-FID chromatogram of the aqueous extract of *Moringa oleifera* seeds showing the separation and retention profiles of the detected phytochemical constituents.

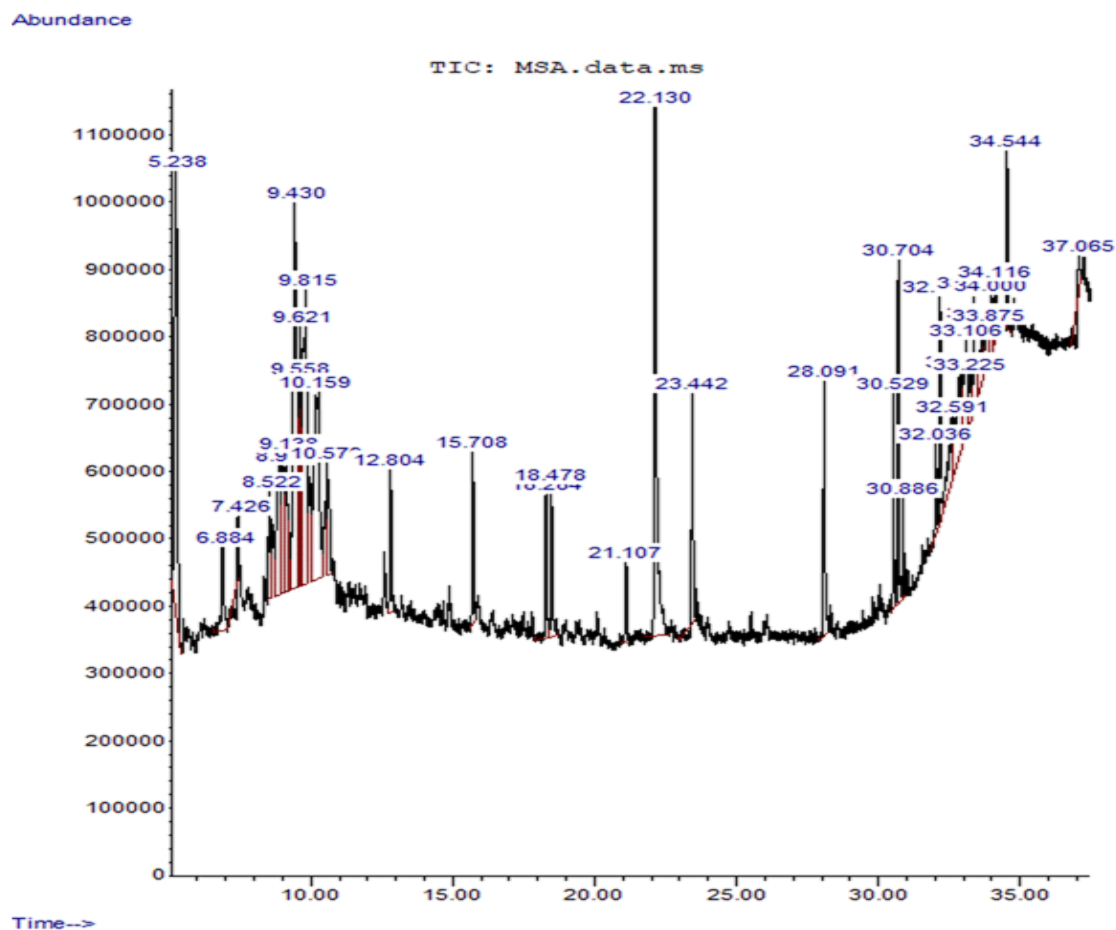


Figure 6: GC-MS chromatogram of the aqueous extract of *Moringa oleifera* seeds showing the separation and retention profiles of the detected phytochemical constituents.

Integrated Phytochemical Interpretation

Collectively, the GC-MS and GC-FID profiles demonstrate a chemically diverse aqueous extract containing phenylpropanoids, phenolic compounds, flavonoids, fatty acids and their derivatives, hydrocarbons, terpenoids, and other related constituents. The two analytical profiles provide complementary information on the composition of the seed extract and reveal the coexistence of several phytochemical classes.

The occurrence of phenolic acids and flavonoids is consistent with the broader literature describing *Moringa oleifera* as a source of chemically diverse bioactive constituents.^{8,11} The structural features of many of the compounds recorded in the present investigation have

previously been associated with antioxidant, redox-modulating, anti-inflammatory, and metabolic activities.^{5,6,9} These reported properties provide biological context for the chemical profile obtained but should not be interpreted as direct demonstration of such activities by the extract.

The present findings therefore primarily establish the phytochemical composition of the aqueous seed extract under the analytical conditions employed. Further experimental studies involving appropriate biological assays would be required to determine whether the identified chemical profile translates into measurable antioxidant, anti-inflammatory, pharmacological, or toxicological effects.

Table 2: GC-FID components of aqueous extract of *Moringa oleifera* seeds

S/N	Compound	Chemical formula	Class	RT	Area (pA·s)	Amount (ppm)
1	Catechin	C ₁₅ H ₁₄ O ₆	Flavonoid; flavan-3-ol	3.925	6.66480	0.929131
2	Apigenin	C ₁₅ H ₁₀ O ₅	Flavonoid; flavone	4.030	320.64548	49.06588
3	Resveratrol	C ₁₅ H ₁₂ O ₃	Stilbene derivative	5.172	20.19850	3.83066
4	Coumaric acid	C ₉ H ₈ O ₃	Hydroxycinnamic acid	5.304	3.00972	0.408988
5	Genistein	C ₁₅ H ₁₀ O ₅	Isoflavone	6.254	15.20751	3.12932
6	Epicatechin	C ₁₅ H ₁₄ O ₆	Flavanol	6.646	13.46197	3.88271
7	Flavone	C ₁₅ H ₁₀ O ₂	Flavonoid	7.004	33.50598	4.70277
8	Daidzein	C ₁₅ H ₁₀ O ₄	Isoflavone	7.219	4.36695	0.605412
9	Epigallocatechin	C ₁₅ H ₁₄ O ₇	Catechin derivative	7.695	1.11425	0.147447

10	Lunamarin	C ₁₈ H ₁₅ NO ₄	Cyanogenic glycoside	7.999	2.90863	0.398732
11	Daidzin	C ₁₇ H ₁₈ O ₇	Isoflavonoid glycoside	8.264	2.98019	0.409889
12	Naringin	C ₂₇ H ₃₂ O ₁₄	Flavanone glycoside	8.401	3.44543	0.474265
13	Flavan-3-ol	C ₁₅ H ₁₄ O ₃	Flavonoid	9.016	3.89771	0.537369
14	Butein	C ₁₅ H ₁₀ O ₄	Flavonoid derivative	9.159	2.13474	0.290926
15	Limonene	C ₁₀ H ₁₈	Monoterpene	9.515	174.46471	25.50425
16	Vanillic acid	C ₈ H ₈ O ₃	Phenolic acid derivative	9.871	3.98630	0.550249
17	Naringenin	C ₁₅ H ₁₂ O ₅	Flavanone	10.156	3.09193	0.425431
18	Luteolin	C ₁₅ H ₁₀ O ₆	Flavonoid; flavone	10.695	1.39242	0.186560
19	Kaempferol	C ₁₅ H ₁₀ O ₆	Flavonoid; flavonol	11.907	2.37106	3.11893
20	Quercetin	C ₁₅ H ₁₀ O ₇	Flavonoid; flavonol	12.738	30.40840	5.26606
21	Myricetin	C ₁₅ H ₁₀ O ₈	Flavonoid; flavonol	14.607	137.45218	15.25054
22	Nobiletin	C ₂₁ H ₂₂ O ₈	Polymethoxylated flavone	15.115	12.05351	4.68398
23	Baicalin	C ₂₁ H ₁₈ O ₁₁	Flavonoid glycoside	17.398	1.19933	0.157748
24	Cinnamic acid	C ₉ H ₈ O ₂	Phenylpropanoid	18.351	1.31555	0.166032
25	Ferulic acid	C ₁₀ H ₁₀ O ₄	Hydroxycinnamic acid	18.617	1.55022	0.204106

RT = retention time; pA·s = picoampere-second; ppm = parts per million.

Conclusion

The present study characterized the phytochemical composition of the aqueous extract of *Moringa oleifera* seeds using GC-MS and GC-FID. GC-MS profiling revealed 35 constituents distributed across phenylpropanoids, phenolic compounds, fatty acids and their derivatives, esters, hydrocarbons, aldehydes, and related chemical classes, while GC-FID profiling detected 25 constituents comprising predominantly flavonoids, phenolic acids, stilbenes, and terpenoid-related compounds. Cinnamic acid, sinapic acid, and 2,4-di-tert-butylphenol were among the prominent GC-MS constituents based on relative peak area, whereas apigenin, limonene, and myricetin were prominent in the GC-FID profile based on the reported concentrations. These findings demonstrate the chemical diversity of the aqueous extract of *Moringa oleifera* seeds and provide a phytochemical profile of its constituents under the analytical conditions employed.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

- Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as potential anti-inflammatory molecules: A review. *Molecules*. 2022; 27(9):2901. Doi: 10.3390/molecules27092901.
- Muscolo A, Sidari M, Settineri G, Papalia T, Mallamaci C. Polyphenols and antioxidant activity: Mechanisms and applications. *Antioxidants*. 2024; 13(8):922. Doi: 10.3390/antiox13080922.
- Xu W, Lu H, Yuan Y, Deng Z, Zheng L, Li H. The antioxidant and anti-inflammatory effects of flavonoids from propolis via Nrf2 and NF-κB pathways. *Foods*. 2022; 11(16):2439. Doi: 10.3390/foods11162439.
- Shahidi F, Zhong Y. Measurement of antioxidant activity in food and biological systems. *J Funct Foods*. 2020; 64:103596. Doi: 10.1016/j.jff.2019.103596.
- Jomova K, Alomar SY, Valko R, Liska J, Nepovimova E, Kuca K, Valko M. Flavonoids and their role in oxidative stress, inflammation, and human diseases. *Chem Biol Interact*. 2025; 413:111489. Doi: 10.1016/j.cbi.2025.111489.
- Xie J, Xiong S, Li Y, Xia B, Li M, Zhang Z, Shi Z, Peng Q, Li C, Lin L, Liao D. Phenolic acids from medicinal plants as anti-inflammatory agents. *Front Immunol*. 2024; 15:1345002. Doi: 10.3389/fimmu.2024.1345002.
- Sies H. Oxidative stress: Concept and some practical aspects. *Antioxidants*. 2020; 9(9):852. Doi: 10.3390/antiox9090852.
- Divya S, Pandey VK, Dixit R, Rustagi S, Suthar T, Atuahene D, Nagy V, Ungai D, Ahmed AEM, Kovacs B, Shaikh AM. Exploring the phytochemical, pharmacological and nutritional properties of *Moringa oleifera*: A comprehensive review. *Nutrients*. 2024; 16(19):3423. Doi: 10.3390/nu16193423.
- Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. *Moringa oleifera* seeds and oil: Characteristics and uses for human health. *Int J Mol Sci*. 2016; 17(12):2141. Doi: 10.3390/ijms17122141.
- Pareek S, Sagar NA, Sharma S, Kumar V, Dey A. *Moringa oleifera*: Nutritional, phytochemical and pharmacological overview. *Front Pharmacol*. 2023; 14:1178923. Doi: 10.3389/fphar.2023.1178923.
- Vergara-Jimenez M, Almatrafi MM, Fernandez ML. Bioactive components in *Moringa oleifera* leaves protect against chronic disease. *Antioxidants*. 2017; 6(4):91. Doi: 10.3390/antiox6040091.
- Saini RK, Sivanesan I, Keum YS. Phytochemicals of *Moringa oleifera*: A review of their nutritional, therapeutic

- and industrial significance. 3 Biotech. 2016; 6(2):203. Doi: 10.1007/s13205-016-0526-3.
13. Khan H, Ullah H, Aschner M, Cheang WS, Akkol EK, Khan A. Neuroprotective effects of flavonoids. *Pharmacol Ther.* 2020; 210:107521. Doi: 10.1016/j.pharmthera.2020.107521.
 14. Huang W, Zhong Y, Gao B, Zheng B, Liu Y. Nrf2-mediated therapeutic effects of dietary flavonoids in different diseases. *Front Pharmacol.* 2023; 14:1240433. Doi: 10.3389/fphar.2023.1240433.
 15. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules.* 2010; 15(10):7313-7352. Doi: 10.3390/molecules15107313.
 16. Niessen WMA. *Liquid chromatography-mass spectrometry.* 3rd ed. Boca Raton: CRC Press; 2017.
 17. Poole CF. *Gas chromatography.* Amsterdam: Elsevier; 2020. Doi: 10.1016/C2016-0-01743-1.
 18. Sparkman OD, Penton Z, Kitson FG. *Gas chromatography and mass spectrometry: A practical guide.* 2nd ed. Amsterdam: Academic Press; 2011.
 19. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants.* 2017; 6(4):42. Doi: 10.3390/plants6040042.
 20. Azwanida NN. A review on extraction methods used in medicinal plants. *Int J Basic Clin Pharmacol.* 2015; 4(3):555-559. Doi: 10.5455/2319-2003.ijbcp20150527.
 21. Lopez-Herrador S, Corral-Sarasa J, Gonzalez-Garcia P, Morillas-Morota Y, Olivieri E, Jimenez-Sanchez L, Diaz-Casado ME. Natural hydroxybenzoic and hydroxycinnamic acids derivatives: Mechanisms of action and therapeutic applications. *Antioxidants.* 2025; 14(6):711. Doi: 10.3390/antiox14060711.
 22. Chen L, Teng H, Jia Z, Battino M, Miron A, Yu Z, Cao H, Xiao J. Intracellular signaling pathways of inflammation modulated by dietary flavonoids: The most recent evidence. *Crit Rev Food Sci Nutr.* 2018; 58(17):2908-2924. Doi: 10.1080/10408398.2017.1345853.