

RESEARCH

Open Access



Moringa oleifera flowers: insights into their aroma chemistry, anti-inflammatory, antioxidant, and enzyme inhibitory properties

Nouran M. Fahmy^{1†}, Shaimaa Fayeze^{1†}, Radwa Wahid Mohamed², Ahmed M. Elissawy^{1,3}, Omayma A. Eldahshan^{1,3*}, Gokhan Zengin⁴ and Abdel Nasser B. Singab^{1,3*}

Abstract

Background *Moringa oleifera* is a highly nutritious plant widely used in traditional medicine.

Results The aroma constituents present in the fresh flowers of *M. oleifera* versus the hydrodistilled oil and hexane extract were studied using GC-MS. Aldehydes were the major class detected in the fresh flowers (64.75%) with *E*-2-hexenal being the predominant component constituting > 50%. Alkane hydrocarbons, monoterpenes, and aldehydes constituted > 50% of the hydrodistilled oil, while alkane hydrocarbons exclusively constitute up to 65.48% of the hexane extract with heptacosane being the major component (46.2%). The cytotoxicity of the hexane extract was assessed on RAW 264.7 macrophages using the MTT assay which revealed no significant cytotoxicity at concentrations of 1 µg/mL and displayed IC₅₀ value at 398.53 µg/mL as compared to celecoxib (anti-inflammatory drug) with IC₅₀ value at 274.55 µg/ml. The hexane extract of *Moringa* flowers displayed good anti-inflammatory activity through suppression of NO, IL-6, and TNF-α in lipopolysaccharide-induced RAW 264.7 macrophages. The total phenolic and flavonoid content in the hexane extract was found to be 12.51 ± 0.28 mg GAE/g extract and 0.16 ± 0.01 mg RuE/g extract, respectively. It displayed moderate antioxidant activity as indicated by the in vitro DPPH, ABTS, CUPRAC, FRAP, and phosphomolybdenum (PBA) assays. No metal chelating properties were observed for the extract. The enzyme inhibitory potential of the hexane extract was evaluated on acetyl- and butyrylcholinesterases (for neuroprotective assessment), α-amylase and α-glucosidase (for antihyperglycemic assessment), and tyrosinase (for dermatoprotective assessment) revealing promising results on cholinesterases, tyrosinase, and α-glucosidase.

Conclusion Our findings suggested that *M. oleifera* leaves can be considered as a multidirectional ingredient for preparing functional applications.

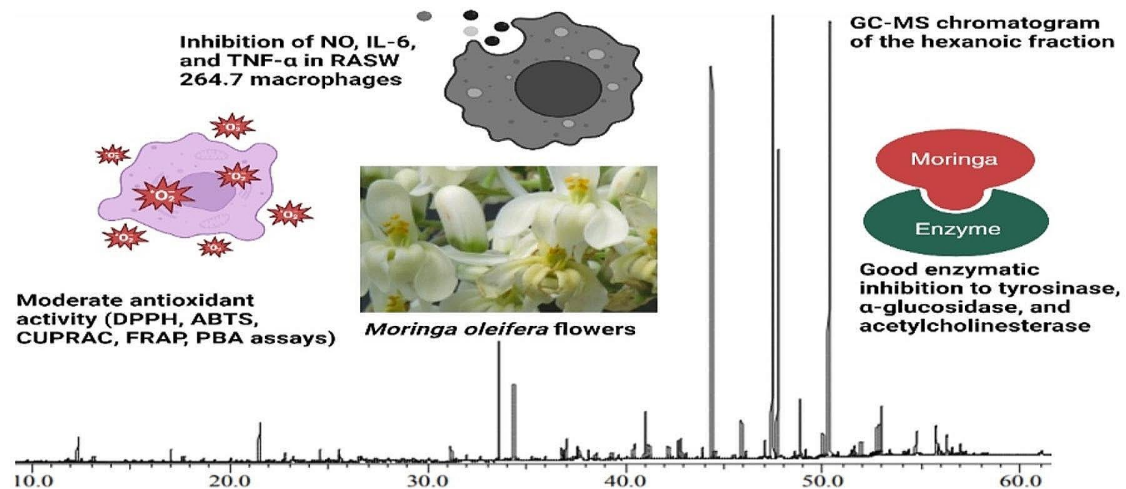
[†]Nouran M. Fahmy and Shaimaa Fayeze contributed equally to this work.

*Correspondence:
Omayma A. Eldahshan
oeldahshan@pharma.asu.edu.eg
Abdel Nasser B. Singab
vpr.nassersingab@asu.edu.eg

Full list of author information is available at the end of the article



Graphical abstract



Keywords *Moringa oleifera* flowers, GC-MS, Essential oil, Antioxidant, Anti-inflammatory, Enzyme inhibition

Introduction

Moringa oleifera Lam. (Moringaceae), also known as the drumstick tree, the horseradish tree, and the ben oil tree, is a highly nutritious edible plant whose leaves and immature seed pods are particularly useful as fortificant in food products with a high safety margin [1–3]. The plant is cultivated worldwide owing to its ability to resist severe drought and mild frost conditions [4]. Traditionally, *Moringa* has been used to treat various health conditions. Its roots were used as a cardiogenic and antiepileptic, while all parts of the plant were utilized to treat high blood pressure, inflammatory diseases, oxidative stress, and infections. The stem bark and flowers were used to regulate blood glucose levels and aid in diabetes management. Leaf extracts are used to treat malnutrition and increase breast milk in nursing mothers as the plant is rich in phytochemicals of steroidal nature. Furthermore, the root bark was utilized in the treatment of lipid disorders [2, 4–6]. The coagulant nature of *Moringa* seeds made them of particular use in water treatment applications and biodiesel production due to their richness in monounsaturated fatty acids like oleic acid [4, 5]. Phytochemical investigations revealed that the leaves are rich sources of flavonoids, ascorbic acid, carotenoids, minerals, vitamins, glucosinolates, and polyphenolic compounds. The flowers comprise amino acids, flavonoids, traces of alkaloids, and minerals. These classes of compounds contribute to a large extent to its documented medicinal uses and

pharmacological activities [4, 5, 7, 8]. Various biological investigations have highlighted the therapeutic potential of *Moringa*. For instance, the flowers and roots have revealed significant hepatoprotective effects. Antimicrobial activities have been reported for roots, bark, leaves, and flowers extracts. Additionally, extracts from *Moringa* leaves and seeds have demonstrated promising antitumor and anti-inflammatory activities [2, 8].

The etiology of many diseases is associated with the excessive formation of free radicals that the body can no longer neutralize, leading to oxidative stress. An increase in oxidative stress can lead to cellular, protein, and DNA damage, resulting in various health problems such as aging, inflammation, diabetes, cancer, neurodegenerative and cardiovascular diseases [9–11].

The inflammatory cascade triggered by injury, infection, or tissue damage is a complex process that led to the stimulation of the immune system and the release of key modulators such as nitric oxide (NO) interleukin 6 (IL-6), and tumor necrosis alpha (TNF- α). NO is a signaling molecule that acts as a vasodilator, and is a crucial step in the inflammatory cascade that facilitates the recruitment of immune cells to the site of injury [12]. IL-6 and TNF are pro-inflammatory cytokines essential for the recruitment and activation of immune cells, amplifying the immune response. Dysregulation or excessive production can contribute to chronic inflammatory conditions and various diseases [13].

On the other hand, enzymes in our body are crucial in catalyzing and regulating many biochemical reactions [14]. Cholinesterase is crucial for maintaining nerve response and function. Inhibitors can be used to improve memory function and treat Alzheimer disease through increasing acetylcholine level in the brain and reduction of β amyloid deposition [15]. Tyrosinase catalyzes the conversion of tyrosine into melanin precursors. In skin-care, tyrosinase inhibitors are often used to treat hyperpigmentation and protect against the harmful effects of UV radiation [16]. In addition, alpha-glucosidase and alpha-amylase facilitate the conversion of starch into more easily absorbable and simplified sugar forms in the body. Consequently, inhibitors can regulate the postprandial increase in blood sugar levels and help in the management of diabetes [17, 18].

Natural products play a vital role in drug discovery owing to their high chemical diversity and unique biological activities. Many of the top successful drugs in clinical use today are derived from natural sources. Natural products have been used in the treatment of various diseases, including infectious and inflammatory diseases, metabolic and cardiovascular disorders, and neurodegenerative conditions [19, 20].

Extensive research has been conducted on the pharmacological activity of *M. oleifera*, yet comprehensive data on the bioactivity and chemical profile of its flowers remains limited. Given the pivotal role of inflammation and oxidative stress in the development of various chronic and life-threatening conditions, added to the significance of enzymes in regulating biochemical reactions in our bodies, there is a need to explore the antioxidant, anti-inflammatory, and enzymatic inhibitory properties of Moringa flower extracts. In the current work, the effect of the extraction technique on the aroma constituents in the fresh flowers of *M. oleifera* compared to the hydrodistilled oil and hexane extract was studied using GC-MS. Additionally, the antioxidant properties, the potential anti-inflammatory activity using mouse macrophage RAW 246.7 cells (which induces the expression of inflammatory mediators), 1 and the enzyme inhibitory activity on cholinesterase, tyrosinase, alpha-amylase, and glycosidase were investigated.

Materials and methods

Plant material and oil extraction

Fresh flowers of *Moringa oleifera* were collected from Faculty of Pharmacy, Ain Shams University, botanical garden in March 2022. The collection of plant material was established in compliance with the national guidelines. Voucher specimens were kept in the Pharmacognosy department herbarium under the code (PHG-P-MO-475). The volatile constituents of *M. oleifera* were characterized using three different extraction

techniques namely, headspace, hydrodistillation, and solvent extraction. Headspace technique was employed on 10 g fresh flowers using Shimadzu headspace sampler HS-20 (Kyoto, Japan), oven temperature was set at 80 °C, equilibrating time was 8 min, and pressurizing time was 2.00 min. Hydrodistillation was done on 100 g fresh flowers using a Clevenger system for 4 h [21]. Solvent extraction was done by macerating 200 g of the flowers in *n*-hexane [22]. The obtained oils were weighed, dried, and stored in air-tight amber and sealed vials at -20 °C till use. The yield was calculated as per 100 g fresh flowers (%w/w). The hydrodistilled oil and the *n*-hexane extract yield was 0.005% and 0.25% w/w, respectively.

GC-MS chromatographic analysis and instrumentation

Gas chromatography coupled to mass spectrometry was carried out utilizing a Shimadzu GC-MS-QP 2010 instrument from Kyoto, Japan, equipped with a DB-1 capillary column (30 m x 0.25 mm i.d. x 0.25 μ m film thickness, provided by Restek, USA). In the case of the hydrodistilled oil, the GC run initiated at 45 °C and maintained this temperature for 2 min. Subsequently, the temperature was programmed to increase to 300 °C at a rate of 5 °C/min and held constant for an additional 10 min. The injector temperature was set at 250 °C, and helium served as the carrier gas at a flow rate of 1.41 ml/min. Samples, with a concentration of 1% v/v, were injected with a 15:1 split ratio, and the injection volume was 1 ml. Ionization energy of 70 eV was used with a scan range of 35–500 atomic mass units [23]. For hexane samples, the measurement conditions were the same, except for the GC program. In this case, the initial temperature was set at 50 °C for 3 min, then raised to 300 °C at a rate of 5 °C/min and maintained at 300 °C for 10 min. Compound identification was achieved by comparing the fragmentation pattern of each compound with NIST-17 library. The retention indices (Kovat index) for each compound were calculated after injecting a standard alkane series (C8-C30) under the same method and conditions. These retention indices were compared with the NIST Chemistry Webbook online database and previously reported retention indices of the identified compounds in the literature [22, 24].

Assessment of the total phenolic and flavonoid contents

The procedure implemented in the determination of the total phenolic and flavonoid contents in the hexane extract of the flowers of *M. oleifera* followed the protocol described by Uysal et al. [25].

In vitro assessment of antioxidant activities of *M. Oleifera* flower hexane extract

All in vitro antioxidant assays were performed following similar protocol as described by Grochowski et al. [26].

The experiments were performed in triplicate and the results were presented as mean and standard deviation.

Cell culture

Murine macrophages (RAW264.7) were obtained from the American Type Culture Collection (ATCC, VA, USA) and were cultured in Dulbecco's modified Eagle medium (DMEM supplemented with high glucose) (Invitrogen/Life Technologies) containing 10% v/v fetal bovine serum (FBS) (Sigma Aldrich Co., St. Louis, MO), penicillin (100 U/mL), and streptomycin (100 µg/mL) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) in a humidified atmosphere with 5% CO₂ at 37 °C. Some cells were sub-cultured for the MTT assay after trypsinization (0.25% trypsin-ethylenediaminetetraacetic acid) (Sigma Aldrich Co., St. Louis, MO), the others were used to measure the production of inflammatory or pre-inflammatory markers such as NO, IL-6, and TNF-α, by addition of *M. oleifera* hexane extract and celecoxib (anti-inflammatory drug).

MTT assay

Moringa hexane extract was dissolved in the least amount of DMSO and subsequently diluted in the culture medium to create the stock solution. RAW264 were cultured in 96-well plates (1.2–1.8 × 10,000 cells/well) in 100 µl growth medium. These cells were then incubated with varying concentrations of *Moringa* extract (1, 4, 16, 63, 250, 1000 µg/mL) for 24 h. DMSO was used as a solvent control. One hundred microliter of 0.5 mg/mL 3-(4,5-dimethylthiazolone-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) (Sigma Aldrich Co., St. Louis, MO) solution was added to each well, and plates were incubated for another 2 h at 37 °C. Medium was removed, 50 µL of dimethyl sulfoxide (Invitrogen/Life Technologies) was added to each well to solubilize formazan crystals and absorbance was then measured at 595 nm (iMark, BioRad, Hercules, CA).

Nitric oxide assay

RAW264 cells were treated with 1 µg/mL of *M. oleifera* hexane extract and celecoxib drug for 24 h. After incubation, the supernatant was collected and used for NO assay. In the case of the NO assay, the supernatants were mixed with Griess reagent containing equal volumes of 1% sulfanilamide (Sigma Aldrich Co., St. Louis, MO) in 2.5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride (Sigma Aldrich Co., St. Louis, MO) solution and then incubated at room temperature for 10 min. The concentration of nitric oxide was measured by OD at 595 nm. Sodium nitrite (NaNO₂) (Sigma Aldrich Co., St. Louis, MO) was used as a standard reagent.

Assessment of the inflammatory mediators IL-6 and TNF-α

RAW264 cells were treated with 1 µg/mL of *M. oleifera* hexane extract and celecoxib drug for 24 h. After incubation, the supernatant was collected and used for cytokine assays. The determination of cytokine concentrations was measured using a Quantikine® enzyme-linked immunosorbent assay kit (R&D Systems, Inc., Minneapolis, MN) for IL-6 and TNF-α following the manufacturer's instructions.

In vitro evaluation of the enzyme inhibition ability of *M. Oleifera* flowers hexane extract

The in vitro enzyme-inhibitory assays were all performed as described by Grochowski et al. [26]. The experiments were performed in triplicate and the results were presented as mean and standard deviation **2.10**.

Statistical analysis

Data were analysed using the Statistical Package for Social Science (SPSS) version 16.0. Statistical differences between groups were performed using one-way analysis of variance (ANOVA). Values are presented as mean ± standard deviation (SD). The mean difference is considered significant at ($p < 0.05$).

Results

GC-MS investigations on the flower volatiles of *Moringa oleifera*

Detailed analysis of the metabolites responsible for the aroma of freshly obtained *Moringa* flowers were studied using GC-MS headspace (Table 1). The oil was further extracted by hydrodistillation and hexane, then the volatiles were similarly analyzed by GC-MS. Differences were observed in the aroma constituents between the fresh and processed flowers (Figs. 1 and 2). *E*-2-Hexenal is an aldehyde compound mostly produced by stressed plants and it is the major metabolite detected in the fresh flowers representing ca. 59.5%. Other less relevant compounds were 2-pentylfuran (7%), 1-hexanol (5%), mentha-6,8-diene (3.3%), and isopropyl isothiocyanate (3.3%).

Isothiocyanates, which are most probably formed by the decomposition of the co-occurring glucosinolates, were only observed in the fresh flowers. In the processed flowers, the oil was dominated by alkanes with tricosane being the major constituent in the hydrodistilled oil (18.5%) while heptacosane being the dominant component in the hexane extract (46.2%). The main metabolites in the fresh and processed flowers are presented in Fig. 1. D-limonene constituted ca. 15% of the GC-MS chromatogram of the hydrodistilled oil yet was totally undetected in the hexane extract. Figure 2 explains the differences in the secondary constituents present in the different oils. It was found that 21 metabolites were solely detected in the hexane extract, 28 metabolites were

Table 1 Analysis of the aromatic components present in *Moringa oleifera* flowers using GC-MS headspace, GC-MS of volatiles obtained by hydrodistillation, and GC-MS of the *n*-hexane extract

Peak no.	t_R (min)	KI_{exp}	KI_{rep}	Identification	Molecular formula	Relative area (%)			Chemical class
						Headspace	Hydrodis-tilled oil	<i>n</i> -hexane extract	
1.	3.58	782	782	Hexanal	C ₆ H ₁₂ O	----	----	0.37	Saturated aldehyde
2.	4.03	800	800	Octane	C ₈ H ₁₈	----	----	0.23	Saturated Alkane
3.	4.43	810	835	Isopropyl isothiocyanate	C ₄ H ₇ NS	3.29	----	----	Isothiocyanate
4.	4.77	823	824	2-Hexenal	C ₆ H ₁₀ O	1.52	----	----	Unsaturated aldehyde
5.	4.90	827	827	(<i>E</i>)-2-Hexenal	C ₆ H ₁₀ O	59.53	----	----	
6.	5.24	840	840	(<i>E</i>)-3-Hexen-1-ol	C ₆ H ₁₂ O	0.73	----	----	Unsaturated alcohol
7.	5.52	850	850	(<i>Z</i>)-2-Hexen-1-ol	C ₆ H ₁₂ O	1.15	----	----	
8.	5.61	853	853	1-Hexanol	C ₆ H ₁₄ O	5.07	----	----	Saturated alcohol
9.	6.24	885	884	Butyl glycol	C ₆ H ₁₄ O ₂	----	----	0.24	Ether derivative
10.	6.87	899	904	2-Butyl isothiocyanate	C ₅ H ₉ NS	1.06	----	----	Isothiocyanate
11.	7.65	927	927	α -Pinene	C ₁₀ H ₁₆	2.85	0.29	----	Cyclic alkanes
12.	8.72	966	966	β -Phellandrene	C ₁₀ H ₁₆	1.54	----	----	
13.	8.81	969	969	β -Pinene	C ₁₀ H ₁₆	1.01	0.23	----	
14.	9.17	983	983	2-Pentylfuran	C ₉ H ₁₄ O	7.04	1.63	----	Furan derivative
15.	9.33	988	988	β -Myrcene	C ₁₀ H ₁₆	----	1.86	----	Unsaturated alkanes
16.	9.39	991	985	<i>trans</i> -2-(2-pentenyl)furan	C ₉ H ₁₂ O	0.70	0.35	----	Furan derivative
17.	9.72	1002	1002	<i>n</i> -Hexyl ethanoate	C ₈ H ₁₆ O ₂	0.74	----	----	Ester derivative
18.	10.03	1012	1017	(+)-4-Carene	C ₁₀ H ₁₆	0.65	----	----	Cyclic alkanes
19.	10.06	1013	1013	α -Terpinene	C ₁₀ H ₁₆	----	0.20	----	
20.	10.14	1016	1016	<i>o</i> -Cymene	C ₁₀ H ₁₄	----	0.46	----	Aromatic compound
21.	10.35	1022	1022	Eucalyptol	C ₁₀ H ₁₈ O	0.99	----	----	Bicyclic ether
22.	10.41	1025	1024	<i>m</i> -Mentha-6,8-diene	C ₁₀ H ₁₆	3.38	----	----	Cyclic alkanes
23.	10.45	1026	1026	D-Limonene	C ₁₀ H ₁₆	----	15.04	----	
24.	10.70	1034	1034	<i>trans</i> - β -Ocimene	C ₁₀ H ₁₆	----	0.62	----	Unsaturated alkane
25.	11.04	1045	1045	<i>cis</i> - β -Ocimene	C ₁₀ H ₁₆	----	0.24	----	
26.	11.30	1054	1054	γ -Terpinene	C ₁₀ H ₁₆	1.52	0.67	----	Cyclic alkane
27.	12.25	1084	1084	Terpinolene	C ₁₀ H ₁₆	----	0.22	----	
28.	12.42	1089	1089	Nonanal	C ₉ H ₁₈ O	----	5.16	0.51	Saturated aldehyde
29.	13.11	1105	1105	Methyl octanoate	C ₉ H ₁₈ O ₂	----	----	0.13	Fatty acid ester
30.	13.68	1130	1127	<i>cis, trans</i> -Nona-2,6-dienal	C ₉ H ₁₄ O	1.15	0.27	----	Unsaturated aldehyde
31.	13.97	1139	1138	<i>trans</i> -2-Nonenal	C ₉ H ₁₆ O	2.55	0.86	----	
32.	14.72	1163	1163	Terpinen-4-ol	C ₁₀ H ₁₈ O	0.65	0.27	----	Cyclic alcohol
33.	15.08	1175	1175	α -Terpineol	C ₁₀ H ₁₈ O	----	0.40	----	
34.	15.30	1182	1182	Hexyl butyrate	C ₁₀ H ₂₀ O ₂	----	0.29	----	Ester derivative
35.	15.65	1193	1200	Dodecane	C ₁₂ H ₂₆	----	0.72	----	Saturated Alkane
36.	15.87	1200	1207	<i>cis</i> -Carveol	C ₁₀ H ₁₆ O	----	0.18	----	Cyclic alcohol
37.	17.04	1233	1234	<i>trans</i> -2-Decenal	C ₁₀ H ₁₈ O	----	----	0.30	Unsaturated aldehyde
38.	17.66	1254	1256	Nonanoic acid	C ₉ H ₁₈ O ₂	----	----	0.22	Fatty acid
39.	19.36	1322	1317	Benzyl Isothiocyanate	C ₈ H ₇ NS	----	0.23	----	Isothiocyanate derivative
40.	20.22	1352	1348	Cinnamic acid methyl ester	C ₁₀ H ₁₀ O ₂	----	0.48	----	Ester derivative
41.	20.59	1365	1364	<i>trans</i> - β -Damascenone	C ₁₃ H ₁₈ O	----	0.34	----	Ketone derivative
42.	22.03	1417	1418	Caryophyllene	C ₁₅ H ₂₄	----	2.05	----	Unsaturated alkane
43.	22.87	1450	1450	Humulene	C ₁₅ H ₂₄	----	2.51	----	
44.	23.29	1467	1468	<i>trans</i> - β -Ionone	C ₁₃ H ₂₀ O	----	0.21	----	Ketone derivative
45.	23.70	1483	1483	β -Selinene	C ₁₅ H ₂₄	----	3.32	----	Cyclic alkane
46.	23.95	1493	1493	α -Selinene	C ₁₅ H ₂₄	----	0.32	----	
47.	24.59	1507	1510	Nonanedioic acid dimethyl ester	C ₁₁ H ₂₀ O ₄	----	----	0.27	Ester derivative
48.	24.69	1521	1517	Kessane	C ₁₅ H ₂₆ O	----	0.38	----	Cyclic ether
49.	25.62	1557	1557	Caryophyllene alcohol	C ₁₅ H ₂₆ O	----	0.59	----	Cyclic alcohol

Table 1 (continued)

Peak no.	t_R (min)	KI_{exp}	KI_{rep}	Identification	Molecular formula	Relative area (%)			Chemical class	
						Headspace	Hydrodis-tilled oil	<i>n</i> -hexane extract		
50.	25.89	1568	1568	Caryophyllene oxide	C ₁₅ H ₂₄ O	----	7.88	----	Epoxy derivative	
51.	26.23	1582	1586	Humulene epoxide I	C ₁₅ H ₂₄ O	----	0.30	----		
52.	26.34	1586	1567	<i>cis</i> -Sesquibabinene hydrate	C ₁₅ H ₂₆ O	----	0.44	----	Cyclic alcohol	
53.	26.49	1592	1593	Humulene epoxide II	C ₁₅ H ₂₄ O	----	3.03	----	Epoxy derivative	
54.	28.56	1681	----	Sedanolid	C ₁₂ H ₁₈ O ₂	----	2.30	----	Cyclic ester (lactone)	
55.	30.07	1746	1740	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	----	0.29	----	Fatty acid	
56.	31.27	1798	1798	Hexadecanal	C ₁₆ H ₃₂ O	----	7.62	----	Saturated aldehyde	
57.	31.97	1832	1830	Hexahydrofarnesyl acetone	C ₁₈ H ₃₆ O	----	0.58	0.12	Ketone derivative	
58.	32.68	1866	1866	Cetyl alcohol	C ₁₆ H ₃₄ O	----	2.98	----	Fatty alcohol	
59.	33.48	1904	1900	Nonadecane	C ₁₉ H ₄₀	----	2.99	1.55	Saturated alkane	
60.	33.63	1902	1902	Hexadecanoic acid methyl ester	C ₁₇ H ₃₄ O ₂	----	----	2.85	Fatty acid ester	
61.	34.43	1950	1950	Palmitic acid	C ₁₆ H ₃₂ O ₂	----	5.21	3.50	Fatty acid	
62.	35.50	2001	1999	Octadecanal	C ₁₈ H ₃₆ O	----	1.62	2.03	Saturated aldehyde	
63.	36.82	2062	2069	Methyl linoleate	C ₁₉ H ₃₄ O ₂	----	----	0.28	Fatty acid ester	
64.	36.89	2065	2058	Methyl linolenate	C ₁₉ H ₃₂ O ₂	----	----	0.20		
65.	37.00	2071	2084	Methyl elaidate	C ₁₉ H ₃₆ O ₂	----	----	0.48		
66.	37.57	2100	2104	Methyl stearate	C ₁₉ H ₃₈ O ₂	----	----	0.27		
67.	37.65	2105	2110	Olealdehyde	C ₁₈ H ₃₄ O	----	----	0.82	Unsaturated aldehyde	
68.	38.16	2132	2137	Stearic acid	C ₁₈ H ₃₆ O ₂	----	----	0.24	Fatty acid	
69.	38.52	2151	2123	Linoleic acid	C ₁₉ H ₃₄ O ₂	----	----	0.19		
70.	39.32	2195	2221	Eicosanal	C ₂₀ H ₄₀ O	----	----	0.75	Saturated aldehyde	
71.	41.04	2305	2300	Tricosane	C ₂₃ H ₄₈	----	18.49	16.58	Saturated alkane	
72.	42.73	2398	2400	Tetracosane	C ₂₄ H ₅₀	----	0.96	0.83		
73.	43.90	2461	----	Lignoceric alcohol	C ₂₄ H ₅₀ O	----	----	1.11	Fatty alcohol	
74.	44.53	2499	2502	Behenic acid	C ₂₃ H ₄₆ O ₂	----	----	0.17	Fatty acid	
75.	47.51	2692	2700	Heptacosane	C ₂₇ H ₅₆	----	----	46.29	Saturated alkane	
76.	47.75	2709	2704	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	----	----	10.05	Aromatic ester	
77.	56.27	3322	3337	β -Amyrenol	C ₃₀ H ₅₀ O	----	----	0.84	Triterpene	
Total identified compounds (%)							97.12	95.08	91.42	
Monoterpenes (%)							10.95	19.83	----	
Oxygenated monoterpenes (%)							1.64	1.88	0.3	
Sesquiterpenes (%)							----	8.2	----	
Oxygenated sesquiterpenes (%)							----	13.2	0.12	

t_R : Retention time, KI_{exp} : Kovat index experimental, KI_{rep} : Kovat index reported

Identification was done based on comparing the mass spectrum and the experimental Kovat index with the reported data on Nist-17 database (National Institute of Standards and Technology) and with previously published data

observed only in the hydrodistilled oil, and 10 components were detected only in the aroma of the fresh flowers. These results prove that processing of the flowers by hydrodistillation or extraction significantly affects the aroma of *Moringa* flowers.

Assessment of the total phenolic and flavonoid content in *Moringa oleifera* flower hexane extract

The total phenolic content in the hexane extract of *M. oleifera* flowers was 12.51 ± 0.28 mg gallic acid equivalent / g extract whereas the total flavonoid was calculated as 0.16 ± 0.01 mg rutin equivalent / g extract. These data

indicate the relatively lower proportion of flavonoids in the total phenolic content of the hexane extract.

Assessment of the antioxidant properties of *Moringa oleifera* flower hexane extract

Antioxidant compounds have the ability to scavenge and, hence, neutralize free radicals owing to the presence of either a phenolic moiety or a conjugated system. The antioxidant activities of the hexane extract of the flowers of *M. oleifera* were quantified as mg trolox equivalent / g extract for DPPH, ABTS, CUPRAC, and FRAP assays with values at 2.71 ± 0.20 , 2.34 ± 0.33 , 32.61 ± 1.22 , and 20.29 ± 0.60 , respectively. No metal chelating abilities

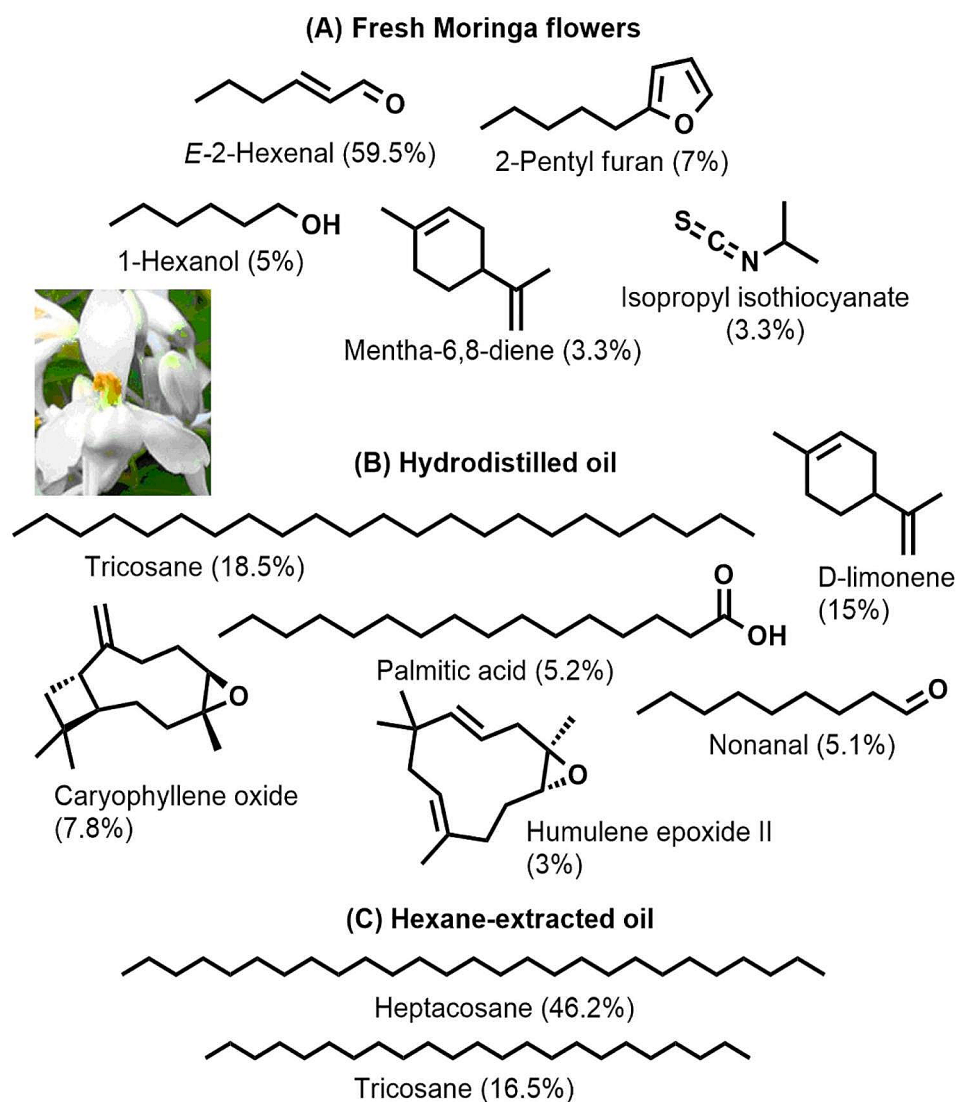


Fig. 1 The structures and percentage of major volatiles detected in the GC-MS chromatograms of **(A)** fresh *Moringa* flowers by headspace and processed flowers by **(B)** hydrodistillation and **(C)** hexane-extraction

were observed for the hexane extract. The phosphomolybdenum assay on the hexane extract showed a value of 0.46 ± 0.03 mmol trolox equivalent / g extract.

Assessment of the effect of *Moringa oleifera* flower hexane extract on the viability of RAW 264.7 macrophages using MTT assay

Cell viability was assessed using the 3-(4,5-dimethylthiazolone-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The viability of cells was reduced in a concentration-dependent manner after adding the hexane extract of the *M. oleifera* flowers at concentrations 1, 4, 6, 63, 250, and 1000 $\mu\text{g}/\text{mL}$ (Fig. 3). The hexane extract did not show significant cytotoxicity at concentrations of 1 $\mu\text{g}/\text{mL}$ and displayed IC_{50} value at 398.53 $\mu\text{g}/\text{mL}$ as compared to celecoxib (anti-inflammatory drug), which showed IC_{50}

value at 274.55 $\mu\text{g}/\text{mL}$. Based on these results, the hexane extract of the *M. oleifera* flowers was prepared at a concentration of 1 $\mu\text{g}/\text{mL}$ and was added to LPS stimulated RAW264.7 macrophages to assess its influence on anti-inflammation.

Assessment of the effect of *Moringa oleifera* flower hexane extract on nitric oxide (NO), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF- α) released from RAW264.7 macrophages

Figure 4 shows the concentration of NO and the pro-inflammatory mediators IL-6 and TNF- α released from RAW264 macrophages into the medium. *M. oleifera* hexane extract and celecoxib (CXB) were significantly lower ($p < 0.05$) than the control (LPS-RAW 264.7 cells), whereas all the mediator concentrations of *M.*

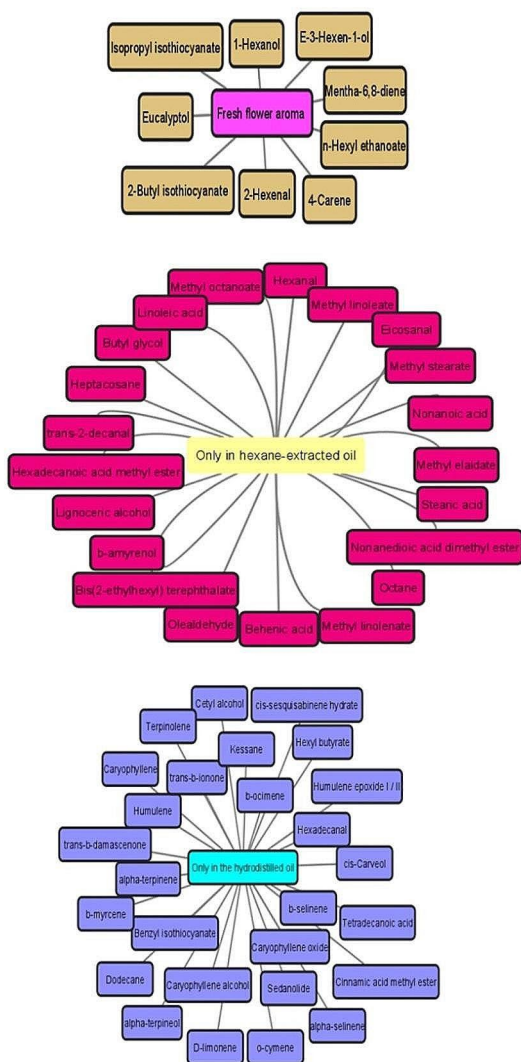


Fig. 2 Schematic representation of the metabolites detected exclusively in fresh *Moringa* flowers, in hexane extracted *Moringa*, and in the hydro-distilled *Moringa* oil

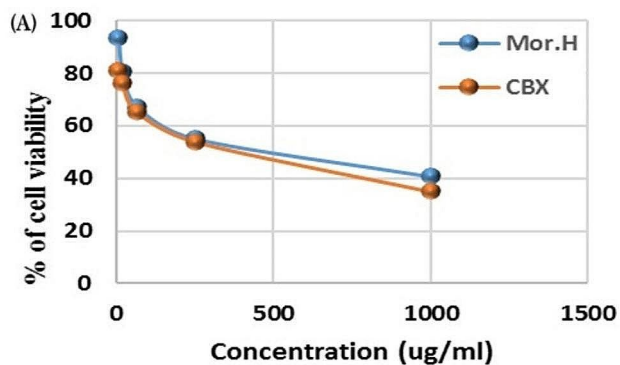


Fig. 3 The effect of the hexane extract of the flowers of *M. oleifera* versus celecoxib (standard) on the percentage viability of RAW 264.7 macrophages

oleifera hexane extract and the control showed a significant increase as compared to the CXB group. These results suggest that the hexane extract of the flowers of *M. oleifera* might show promising anti-inflammatory activity.

Assessment of the enzyme-inhibitory properties of *Moringa oleifera* flower hexane extract

Assessment of the neuroprotective effects of the hexane extract of *M. oleifera* flowers through suppressing the activity of acetyl- (AChE) and butyrylcholinesterases (BChE) was performed. Results showed that the inhibitory activities of the extract to AChE and BChE were of values 2.55 ± 0.01 and 2.74 ± 0.35 mg galantamine equivalent / g extract, respectively.

The dermo-protective potential of the extract was assessed by measuring its inhibition capacity to tyrosinase, a key enzyme involved in melanin synthesis, which was found to be of value 38.32 ± 0.73 mg equivalent of kojic acid / g extract.

The antihyperglycemic activity of the extract was evaluated by measuring its suppression ability to amylase and glucosidase enzymes involved in carbohydrate digestion. Their inhibition values were 0.40 ± 0.01 and 2.36 ± 0.01 mg acarbose equivalent / g extract for amylase and glucosidase, respectively.

Discussion

Genus *Moringa* includes 13 species, of which *M. oleifera* is widely cultivated worldwide due to its richness in essential nutrients found in all its parts [27]. GC-MS-assisted studies on fresh flowers, hydrodistilled oil, and hexane extract resulted in the identification of a total of 97.12%, 95.08%, and 91.42% of metabolites, respectively.

Fresh flowers harbour several chemical classes including aldehydes (64.75%), monoterpenes (10.95%), aliphatic and terpene alcohols (8.59%), furan derivatives (7.74%), isothiocyanates (4.36%), and esters (0.74%). The major compound detected in fresh *Moringa* flowers which is eventually responsible for the floral aroma was *E*-2-hexenal, which represented 59.5% of the total GC-MS chromatogram. No fatty acids, alkane hydrocarbons, sesquiterpenes, ketones, or ethers were detected in fresh *Moringa* flowers.

In the hydrodistilled oil of *M. oleifera* flowers, the distribution and relative percentage of chemical classes were different with linear alkanes representing 23.16% followed by monoterpenes (19.83%), aldehydes (15.53%), epoxy derivatives and cyclic ethers (11.59%), sesquiterpenes (8.2%), fatty acids (5.5%), alcohols (4.42%), esters (3.07%), and ketones (1.13%). Tricosane, D-limonene, caryophyllene oxide, nonanal, and palmitic acid constituted 51.6% of *Moringa* hydrodistilled oil. The isothiocyanate content was dramatically reduced in the

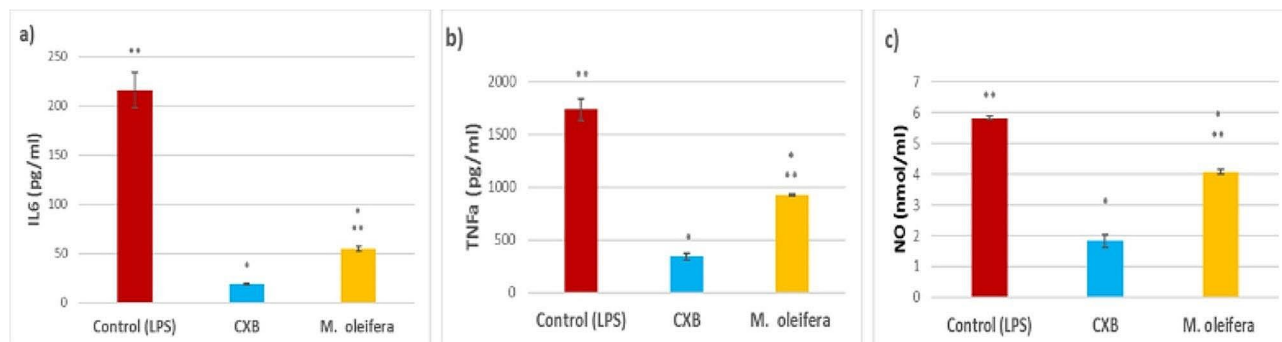


Fig. 4 Assessment of (a) IL-6; (b) TNF- α ; and (c) NO concentrations after treatment with *M. oleifera* hexane extract and celecoxib standard (CXB). Data are shown as mean \pm SD. *indicated a significant decrease as compared to the control (lipopolysaccharide LPS) group. **indicated a significant increase as compared to the CXB group

hydrodistilled oil reaching 0.23% of the total content. The hydrodistilled oil was the only one which contain sesquiterpenes.

In the hexane extracted *Moringa* flowers, alkane hydrocarbons constituted the major class representing ca. 65.48% followed by aliphatic and fatty esters (14.53%), fatty acids (4.32%), aldehydes (3.96%), alcohols (1.35%), and ketones (0.12%). The main fatty acid detected in the hydrodistilled oil and hexane extract was palmitic acid with major predominance in the former. Esters of polyunsaturated fatty acids like linoleic and linolenic acids were exclusively detected in the hexane extract. Furan derivatives, isothiocyanates, mono- and sesquiterpenes along with epoxy and cyclic ethers were absent in the hexane extract.

Having a detailed look at the literature, and to the best of our knowledge, very few data handled the chemical profiling of *Moringa* flowers. Previous GC-MS study on the chloroform fraction of *Moringa* flowers focused mainly on their fatty acid profile with predominance of palmitic acid as the main saturated fatty acid and linoleic and linolenic acids as the main polyunsaturated fatty acids [28]. These data were consistent with that presented in the current study where the aforementioned fatty acids were all observed in the hexane extract, yet only palmitic acid was detected in the hydrodistilled oil. Another study [29] on the hydrodistilled flower oil obtained from *Moringa oleifera* cultivated in Portugal revealed certain consistency with our study in the prevalence of alkane hydrocarbons and palmitic acid in the oil, yet the main hydrocarbons detected in the Portuguese oil were heptacosane, nonacosane, and pentacosane however in the Egyptian oil it was tricosane as the main saturated alkane hydrocarbon detected. The hydrodistilled oil of *Moringa* flowers cultivated in Cuba [30] showed significant differences in the relative percentage of aroma constituents where the Havanan oil contains *E*-nerolidol (13.4%), α -terpineol (7.8%), benzyl isothiocyanate (6.4%), *Z*-3-hexenol (5.7%), 2-methylbutan-1-ol (5.6%), limonene

(5.4%), linalool (4.1%), nonanal (4.1%), isopropyl isothiocyanate (3.9%), hexanal (2.4%), *E*-2-hexenal (2.4%), and *E*-2-hexenol (2.3%). In Egyptian hydrodistilled oil, *E*-nerolidol was not observed. Isopropyl isothiocyanate, *E*-3-hexenol, *Z*-2-hexenol, and *E*-2-hexenal were only detected in the fresh, rather than the hydrodistilled, flowers. Limonene content constituted up to 15.04% (rather than 5.4% as observed in the Havanan oil) while α -terpineol and benzyl isothiocyanate (0.23%) were only present in traces (0.40%) in the Egyptian oil. These differences could be due to climatic variations, soil properties, and agricultural conditions which may contribute to changes in the relative percentage of chemical aroma constituents produced by the plant.

Several medicinal and pharmacological effects have been reported on *Moringa oleifera* in particular on the leaves and roots, but comprehensive data on the bioactivity of the flowers are still lacking. As inflammation is a key player that mediate the pathogenesis of many chronic, and sometimes lethal, diseases like cancer, diabetes, cardiovascular, and autoimmune disorders, it is worthy to investigate the anti-inflammatory properties of the flower extract specially since other *Moringa* parts have been shown to display potent anti-inflammatory activity in vitro and in vivo [31–38] which might be attributed to their content of phenolics [39], flavonoids, peptides [40], polysaccharides [41], and/or isothiocyanates [42].

The total phenolic content in the hexane extract of *Moringa* flowers was 12.51 ± 0.28 mg GAE/g extract. This value is actually 3x higher than the total phenolic content in the hexane extract of the leaves which was reported to be 3.85 ± 0.60 mg GAE/g extract [35]. The hexane extract of *Moringa* flowers collected from India showed total phenolic content of 33.08 ± 0.1 mg GAE/g extract which was higher than that in the aqueous and methanol extracts yet lower than the acetone and ethyl acetate fractions [43]. However, the total flavonoid content in the hexane extract of *Moringa* flowers was found

to be significantly low reaching 0.16 ± 0.01 mg RuE/g extract compared to the total flavonoid content in the hexane extract of the leaves which was reported to be 2.83 ± 0.74 mg catechin equivalent/g extract [35]. Consistent with our study, the total flavonoid content reported in the hexane extract of the Indian *Moringa* flowers was the lowest (at value 2.31 ± 0.15 mg catechin equivalent/g) compared to ethyl acetate or methanol extracts [43]. In general, the total phenolic and flavonoid content in polar extracts like alcohol or water is much higher than non-polar solvents like ether and hexane [35].

The antioxidant properties of *Moringa* flowers hexane extract were assessed in vitro using DPPH, ABTS, CUPRAC, FRAP, metal chelating assay, and phosphomolybdenum assay. The extract displayed activity corresponding to 2.71 ± 0.2 mg trolox equivalent (for DPPH), 2.34 ± 0.3 mg TE/g (for ABTS), 32.61 ± 1.2 mg TE/g (for CUPRAC), and 20.2 ± 0.6 mg TE/g (for FRAP). Compared to the reported data on the DPPH (from 29 to 35 μ M TE/g) and ABTS assays (from 7 to 29 μ M TE/g) on the polar (i.e., methanol and water) extracts of *Moringa oleifera* seeds [44], the hexane fraction of *M. oleifera* flowers showed low antioxidant potential owing to its very low content of flavonoids. Despite previous reports on the metal chelating properties of the polyphenol rich fraction of *M. oleifera* leaves [45], we did not observe any metal chelating effect for the hexane extract of the flowers.

The effect of *Moringa* hexane extract on suppressing the release of nitric oxide (a signalling molecule that plays a key role in the pathogenesis of inflammation), interleukin-6 (a proinflammatory cytokine), and tumor necrosis factor alpha (an inflammatory cytokine produced by macrophages during acute inflammation) was assessed in lipopolysaccharide-induced RAW 264.7 macrophages. Compared to the control (LPS-stimulated murine macrophages), the *Moringa* hexane extract and celecoxib reduced NO production significantly by 30% and 68.6% respectively. Furthermore, *Moringa* hexane extract and celecoxib reduced the release of IL-6 significantly by 46.8% and 80.2% respectively, whereas *Moringa* hexane extract and celecoxib reduced the release of TNF- α significantly by 74.7% and 91.3% respectively. Despite the higher efficacy of the non-steroidal anti-inflammatory drug celecoxib than *Moringa* extract, still the latter display higher safety margin with moderate anti-inflammatory effect. Our results are consistent with those previously described on the anti-inflammatory properties of *Moringa* flowers, yet these studies were conducted on the alcoholic extract [46, 47].

Several reports have described enzyme inhibitory potential for *Moringa* although the majority of studies were conducted on the leaves [48–57]. Enzymes involved in carbohydrate digestion like α -amylase and α -glucosidase have been reported to be inhibited by

Moringa oleifera leaves [53] and seeds [58], also the leaves could inhibit pancreatic cholesterol esterase, which is involved in the control of blood lipids [48]. The hexane extract of *Moringa* roots have been reported to display optimal α -glucosidase and α -amylase which might justify its efficacy as antihyperglycemic remedy [59]. These results are consistent with that presented in the current study about the inhibitory potential of the hexane extract of *Moringa* flowers on α -amylase and α -glucosidase yet with greater potential to inhibit the later enzyme. The hexane extract of *Moringa* flowers displayed neuroprotective potential in patients with Alzheimer due to its inhibition to acetylcholinesterase involved in the breakdown of the neurotransmitter acetylcholine. The ability of the hexane extract of *Moringa* flowers to inhibit tyrosinase, a key enzyme involved in melanin synthesis, make it of potential use in the management of skin depigmentation disorders and could be incorporated among the constituents of a natural remedy for skin whitening. Tyrosinase exhibits a dual role in Parkinson's disease, serving both as a catalyst for dopamine synthesis crucial for neurotransmission and as a catalyst for the conversion of dopamine to reactive oxygen species (ROS) and toxic metabolites. This process contributes to oxidative stress and neurodegeneration. Thus, its inhibition can also be considered as a neuroprotective possibility [60].

Previous studies showed that undecanoic acid produced by *Aspergillus flavus* (an endophyte on *M. oleifera*) inhibit to high extent tyrosinase enzyme [61]. Another study showed that luteolin might be the major contributor to tyrosinase inhibition as confirmed by its inhibition kinetics [57].

Conclusion

The aroma of fresh *Moringa oleifera* flowers is mainly attributed to its content of *E*-2-hexenal which constitute more than 50% of its total GC-MS chromatogram. Meanwhile the aroma of the hydrodistilled oil might be attributed to its D-limonene content. Hexane extract is dominated with alkane hydrocarbons like heptacosane and tricosane. Cell viability assay showed that *M. oleifera* hexane extract is not cytotoxic to RAW 264.7 macrophages at concentrations of 1 μ g/mL *Moringa oleifera* flowers hexane extract reduced the production of NO as well as the proinflammatory mediators IL-6 and TNF- α in LPS-stimulated RAW 264.7 macrophages. The extract displayed moderate antioxidant activity yet good enzymatic inhibition especially to acetylcholinesterase, tyrosinase, and α -glucosidase. The good anti-inflammatory and enzyme inhibitory properties of the hexane extract of *M. oleifera* flowers beside its non-cytotoxic nature to cells warrant further investigations to explore the extract's mechanisms of action and potential therapeutic applications.

Acknowledgements

The authors would like to acknowledge the Science and Technology and Innovation fund (STIFA) for funding of research project number 46667 under the title "Sustainability of lab. Capacities of Canter of Drug Discovery Research and Development".

Author contributions

Shaimaa Fayez: Essential oil extraction, GC-MS interpretation, writing the original draft
Nouran Fahmy: Essential oil extraction, GC-MS interpretation, writing the original draft
Radwa Wahid: Anti-inflammatory assay, writing the original draft
Gokhan Zengin: Antioxidant and enzyme inhibitory assay
Ahmed Elissawy: manuscript revision
Omayma Eldahshan: supervision, manuscript revision
Abdel Nasser Singab: conceptualization, supervision, manuscript revision.

Funding

Open access funding is provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Data availability

Data are available upon reasonable request from the first author; Nouran_Fahmy@pharma.asu.edu.eg.

Declarations

Ethics approval and consent to participate

Fresh flowers of *Moringa oleifera* were collected from Faculty of Pharmacy, Ain Shams University, botanical garden in March 2022. The collection of plant material was established in compliance with the national guidelines. The plant was authenticated by Professor Usama K. Abdel Hameed, Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt. Voucher specimens were kept in the Pharmacognosy department herbarium under the code (PHG-P-MO-475).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt

²Department of Biochemistry and Nutrition, Women's College for Arts Science and Education, Ain Shams University, Cairo, Egypt

³Center for Drug Discovery Research and Development, Ain Shams University, Cairo 11566, Egypt

⁴Department of Biology, Science Faculty, Selcuk University, Konya 42130, Türkiye

Received: 9 January 2024 / Accepted: 3 July 2024

Published online: 26 July 2024

References

1. Stohs SJ, Hartman MJ. Review of the Safety and Efficacy of *Moringa oleifera*. *Phytother Res*. 2015;29(6):796–804. <https://doi.org/10.1002/ptr.5325>.
2. Bhattacharya A, Tiwari P, Sahu PK, Kumar S. A review of the Phytochemical and pharmacological characteristics of *Moringa oleifera*. *J Pharm Bioallied Sci*. 2018;10(4):181–91. https://doi.org/10.4103/jpbs.jpbs_126_18.
3. Oyeyinka AT, Oyeyinka SA. *Moringa oleifera* as a food fortificant: recent trends and prospects. *J Saudi Soc Agric Sci*. 2018;17(2):127–36. <https://doi.org/10.1016/j.jssas.2016.02.002>.
4. Gopalakrishnan L, Doriya K, Kumar DS. *Moringa oleifera*: a review on nutritive importance and its medicinal application. *Food Sci Hum Wellness*. 2016;5(2):49–56. <https://doi.org/10.1016/j.fshw.2016.04.001>.
5. Saini RK, Sivanesan I, Keum Y-S. Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. *Biotech*. 2016;6(2). <https://doi.org/10.1007/s13205-016-0526-3>.
6. Mishra G, Singh P, Verma R, Kumar S, Srivastav S, Jha KK, Khosa RL. Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: an overview. *Pharm Lett*. 2011;3(2):141–64.
7. Anwar F, Latif S, Ashraf M, Gilani AH. *Moringa oleifera*: a food plant with multiple medicinal uses. *Phytother Res*. 2007;21(1):17–25. <https://doi.org/10.1002/ptr.2023>.
8. Toma A, Deyno S. Phytochemistry and pharmacological activities of *Moringa oleifera*. *Int J Pharmacognosy*. 2014;1(4):222–31.
9. Preiser JC. Oxidative stress. *J Parenter Enter Nutr*. 2012;36(2):147–54.
10. Teleanu DM, Niculescu A-G, Lungu II, Radu CI, Vladăcenco O, Roza E, Costăchescu B, Grumezescu AM, Teleanu RI. An overview of oxidative stress, Neuroinflammation, and neurodegenerative diseases. *Int J Mol Sci*. 2022;23(11). <https://doi.org/10.3390/ijms23115938>.
11. Abd El-Ghffar EA, Al-Sayed E, Shehata SM, Eldahshan OA, Efferth T. The protective role of *Ocimum basilicum* L.(Basil) against aspirin-induced gastric ulcer in mice: impact on oxidative stress, inflammation, motor deficits and anxiety-like behavior. *Food Funct*. 2018;9(8):4457–68.
12. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*. 2014;6(10):a016295.
13. Sharma JN, Al-Omran A, Parvathy SS. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*. 2007;15(6):252–9. <https://doi.org/10.1007/s10787-007-0013-x>.
14. Kaur R, Sekhon BS. Enzymes as drugs: an overview. *J Pharm Educ Res* 3(2) (2012).
15. Anand P, Singh B. A review on cholinesterase inhibitors for Alzheimer's disease. *Arch Pharm Res*. 2013;36:375–99.
16. Chang T-S. An updated review of tyrosinase inhibitors. *Int J Mol Sci*. 2009;10(6):2440–75.
17. Sun L, Warren FJ, Gidley MJ. Natural products for glycaemic control: polyphenols as inhibitors of alpha-amylase. *Trends Food Sci Technol*. 2019;91:262–73.
18. Fahmy NM, Faye S, Uba AI, Shariati MA, Aljohani ASM, El-Ashmawy IM, Batiha GE, Eldahshan OA, Singab AN, Zengin G. Comparative GC-MS analysis of Fresh and dried *Curcuma* essential oils with insights into their antioxidant and enzyme inhibitory activities. *Plants*. 2023;12(9). <https://doi.org/10.3390/plants12091785>.
19. Liu H, Xu T, Xue Z, Huang M, Wang T, Zhang M, Yang R, Guo Y. Current development of thiazole-containing compounds as potential antibacterials against methicillin-resistant staphylococcus aureus. *ACS Infect Dis*. 2024;10(2):350–70.
20. Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. *Metabolites*. 2012;2(2):303–36.
21. Shahat EA, Bakr RO, Eldahshan OA, Ayoub NA. Chemical composition and biological activities of the essential oil from leaves and flowers of *Pulicaria incisa* sub. *Candolleana* (family Asteraceae). *Chem Biodivers*. 2017;14(4):e1600156.
22. Singab ANB, Mostafa NM, Eldahshan OA, Ashour ML, Wink M. Profile of volatile components of hydrodistilled and extracted leaves of *Jacaranda acutifolia* and their antimicrobial activity against foodborne pathogens. *Nat Prod Commun*. 2014;9(7):1934578X1400900731.
23. Torky ZA, Moussa AY, Abdelghffar EA, Abdel-Hameed UK, Eldahshan OA. Chemical profiling, antiviral and antiproliferative activities of the essential oil of *Phlomis aurea* Decne grown in Egypt. *Food Funct*. 2021;12(10):4630–43.
24. Eldahshan OA, Halim AF. Comparison of the composition and antimicrobial activities of the essential oils of green branches and leaves of Egyptian navel orange (*Citrus sinensis* (L.) Osbeck var. Malesy). *Chem Biodivers*. 2016;13(6):681–5.
25. Uysal S, Zengin G, Locatelli M, Bahadori MB, Mocan A, Bellagamba G, De Luca E, Mollica A, Aktumsek A. Cytotoxic and enzyme inhibitory potential of two *Potentilla* species (*P. Speciosa* L. and *P. reptans* Willd.) And their chemical composition. *Front Pharmacol*. 2017;8(290). <https://doi.org/10.3389/fphar.2017.00290>.
26. Grochowski DM, Uysal S, Aktumsek A, Granica S, Zengin G, Ceylan R, Locatelli M, Tomczyk M. In vitro enzyme inhibitory properties, antioxidant activities, and phytochemical profile of *Potentilla thuringiaca*. *Phytochem Lett*. 2017;20:365–72.
27. Dhakad AK, Ikram M, Sharma S, Khan S, Pandey VV, Singh A. Biological, nutritional, and therapeutic significance of *Moringa oleifera* Lam. *Phytother Res*. 2019;33(11):2870–903. <https://doi.org/10.1002/ptr.6475>.

28. Saini RK, Shetty NP, Giridhar P. GC-FID/MS Analysis of Fatty Acids in Indian cultivars of *Moringa oleifera*: potential sources of PUFA. *J Am Oil Chem Soc*. 2014;91(6):1029–34. <https://doi.org/10.1007/s11746-014-2439-9>.
29. Monteiro J, Scotti-Campos P, Pais I, Figueiredo AC, Viegas D, Reboredo F. Elemental composition, total fatty acids, soluble sugar content and essential oils of flowers and leaves of *Moringa oleifera* cultivated in Southern Portugal. *Heliyon*. 2022;8(12):e12647. <https://doi.org/10.1016/j.heliyon.2022.e12647>.
30. Pino JA. Floral Scent composition of *Moringa oleifera* Lam. *J Essent Oil-Bear Plants*. 2013;16(3):315–7. <https://doi.org/10.1080/0972060X.2013.813189>.
31. Ndiaye M, Dieye AM, Mariko F, Tall A, Sall Diallo A, Faye B. Contribution to the study of the anti-inflammatory activity of *Moringa oleifera* (moringaceae). *Dakar Med*. 2002;47(2):210–2.
32. Martínez-González CL, Martínez L, Martínez-Ortiz EJ, González-Trujano ME, Déciga-Campos M, Ventura-Martínez R, Díaz-Reval I. *Moringa oleifera*, a species with potential analgesic and anti-inflammatory activities. *Biomed Pharmacother*. 2017;87:482–8. <https://doi.org/10.1016/j.biopha.2016.12.107>.
33. Coppin JP, Xu Y, Chen H, Pan M-H, Ho C-T, Juliani R, Simon JE, Wu Q. Determination of flavonoids by LC/MS and anti-inflammatory activity in *Moringa oleifera*. *J Funct Foods*. 2013;5(4):1892–9. <https://doi.org/10.1016/j.jff.2013.09.010>.
34. Xu Y-B, Chen G-L, Guo M-Q. Antioxidant and anti-inflammatory activities of the crude extracts of *Moringa oleifera* from Kenya and their correlations with flavonoids. *Antioxidants*. 2019;8(8). <https://doi.org/10.3390/antiox8080296>.
35. Saleem A, Saleem M, Akhtar MF. Antioxidant, anti-inflammatory and antiarthritic potential of *Moringa oleifera* Lam: an ethnomedicinal plant of Moringaceae family. *S Afr J Bot*. 2020;128:246–56. <https://doi.org/10.1016/j.sajb.2019.11.023>.
36. Sulaiman MR, Zakaria ZA, Bujarimin AS, Somchit MN, Israf DA, Moin S. Evaluation of *Moringa oleifera* Aqueous Extract for Antinociceptive and anti-inflammatory activities in animal models. *Pharm Biol*. 2008;46(12):838–45. <https://doi.org/10.1080/13880200802366710>.
37. Araújo LCC, Aguiar JS, Napoleão TH, Mota FVB, Barros ALS, Moura MC, Coriolano Marília C, Coelho LCBB, Silva TG, Paiva PMG. Evaluation of cytotoxic and anti-inflammatory activities of extracts and lectins from *Moringa oleifera* Seeds. *PLoS ONE*. 2013;8(12):e81973. <https://doi.org/10.1371/journal.pone.0081973>.
38. Minaiyan M, Asghari G, Taheri D, Saeidi M, Nasr-Esfahani S. Anti-inflammatory effect of *Moringa oleifera* Lam. Seeds on acetic acid-induced acute colitis in rats. *Avicenna J Phytomed*. 2014;4(2):127–36.
39. Cheenpracha S, Park E-J, Yoshida WY, Barit C, Wall M, Pezzuto JM, Chang LC. Potential anti-inflammatory phenolic glycosides from the medicinal plant *Moringa oleifera* fruits. *Bioorg Med Chem*. 2010;18(17):6598–602. <https://doi.org/10.1016/j.bmc.2010.03.057>.
40. Avilés-Gaxiola S, León-Félix J, Jiménez-Nevárez YB, Angulo-Escalante MA, Ramos-Payán R, Colado-Velázquez J, Heredia JB. Antioxidant and anti-inflammatory properties of novel peptides from *Moringa oleifera* Lam. *Leaves*. *S Afr J Bot*. 2021;141:466–73. <https://doi.org/10.1016/j.sajb.2021.05.033>.
41. Cui C, Chen S, Wang X, Yuan G, Jiang F, Chen X, Wang L. Characterization of *Moringa oleifera* roots polysaccharide MRP-1 with anti-inflammatory effect. *Int J Biol Macromol*. 2019;132:844–51. <https://doi.org/10.1016/j.ijbiomac.2019.03.210>.
42. Jaja-Chimedza A, Graf BL, Simmler C, Kim Y, Kuhn P, Pauli GF, Raskin I. Biochemical characterization and anti-inflammatory properties of an isothiocyanate-enriched moringa (*Moringa oleifera*) seed extract. *PLoS ONE*. 2017;12(8):e0182658. <https://doi.org/10.1371/journal.pone.0182658>.
43. Punia J, Singh R. Antioxidant potential and nutritional content of stem, bark and pod of Drumstick tree (*Moringa oleifera* Lam.) From semi-arid region of Haryana. *J Indian Chem Soc*. 2017;94:103–10.
44. Adebayo IA, Arsad H, Samian MR. Total phenolics, total flavonoids, antioxidant capacities, and Volatile Compounds Gas Chromatography-Mass Spectrometry profiling of *Moringa oleifera* Ripe seed Polar fractions. *Pharmacogn Mag*. 2018;14(54):191–4. https://doi.org/10.4103/pm_pm_212_17.
45. Verma AR, Vijayakumar M, Mathela CS, Rao CV. In vitro and in vivo antioxidant properties of different fractions of *Moringa oleifera* leaves. *Food Chem Toxicol*. 2009;47(9):2196–201. <https://doi.org/10.1016/j.fct.2009.06.005>.
46. Tan WS, Arulselvan P, Karthivashan G, Fakurazi S. *Moringa oleifera* Flower Extract Suppresses the Activation of Inflammatory Mediators in Lipopolysaccharide-Stimulated RAW 264.7 Macrophages via NF B Pathway. *Mediators Inflamm*. 2015;720171 (2015) <https://doi.org/10.1155/2015/720171>.
47. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, in-vitro antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. *Asian Pac J Trop Biomed*. 2013;3(8):623–7. [https://doi.org/10.1016/S2221-1691\(13\)60126-4](https://doi.org/10.1016/S2221-1691(13)60126-4).
48. Adisakwattana S, Chanathong B. Alpha-glucosidase inhibitory activity and lipid-lowering mechanisms of *Moringa oleifera* leaf extract. *Eur Rev Med Pharmacol Sci*. 2011;15(7):803–8.
49. Khan H, Jaiswal V, Kulshreshtha S, Khan A. Potential angiotensin converting enzyme inhibitors from *Moringa oleifera*. *Recent Pat Biotechnol*. 2019;13(3):239–48. <https://doi.org/10.2174/187220831366619021114229>.
50. Natsir H, Wahab AW, Laga A, Arif AR. Inhibitory activities of *Moringa oleifera* leaf extract against α -glucosidase enzyme in vitro. *J Phys: Conference Series*. 979(1),012019 (2018) <https://doi.org/10.1088/1742-6596/979/1/012019>.
51. Francis JA, Jayaprakasam B, Olson LK, Nair MG. Insulin secretagogues from *Moringa oleifera* with cyclooxygenase enzyme and lipid peroxidation inhibitory activities. *Helv Chim Acta*. 2004;87(2):317–26. <https://doi.org/10.1002/hlca.200490029>.
52. Bijina B, Chellappan S, Krishna JG, Basheer SM, Elyas KK, Bahkali AH, Chandrasekaran M. Protease inhibitor from *Moringa oleifera* with potential for use as therapeutic drug and as seafood preservative. *Saudi J Biol Sci*. 2011;18(3):273–81. <https://doi.org/10.1016/j.sjbs.2011.04.002>.
53. Ademiluyi AO, Aladeselu OH, Oboh G, Boligon AA. Drying alters the phenolic constituents, antioxidant properties, α -amylase, and α -glucosidase inhibitory properties of *Moringa (Moringa oleifera)* leaf. *Food Sci Nutr*. 2018;6(8):2123–33. <https://doi.org/10.1002/fsn3.770>.
54. Bijina B, Chellappan S, Basheer SM, Elyas KK, Bahkali AH, Chandrasekaran M. Protease inhibitor from *Moringa oleifera* leaves: isolation, purification, and characterization. *Process Biochem*. 2011;46(12):2291–300. <https://doi.org/10.1016/j.procbio.2011.09.008>.
55. Pontual EV, de Lima Santos ND, de Moura MC, Coelho LCBB, do Amaral Ferraz Navarro DM, Napoleão TH, Paiva PMG. Trypsin inhibitor from *Moringa oleifera* flowers interferes with survival and development of *Aedes aegypti* larvae and kills bacteria inhabitant of larvae midgut. *Parasitol Res*. 113(2),727–733 (2014) <https://doi.org/10.1007/s00436-013-3702-y>.
56. Ogundipe A, Adetuyi B, Iheagwam F, Adefoyeke K, Olugbuyiro J, Ogunlana O, Ogunlana O. *In Vitro* Experimental Assessment of Ethanolic Extract of *Moringa oleifera* Leaves as an α Amylase and a Lipase Inhibitor. *Biochem Res Int*. 2022;4613109 (2022) <https://doi.org/10.1155/2022/4613109>.
57. Karray A, Alonazi M, Jallouli R, Alanazi H, Ben Bacha A. A proteinaceous α -amylase inhibitor from *Moringa Oleifera* Leaf Extract: purification, characterization, and Insecticide effects against *C. maculatus* Insect Larvae. *Molecules*. 2022;27(13). <https://doi.org/10.3390/molecules27134222>.
58. Barrientos RE, Ahmed S, Cortés C, Fernández-Galleguillos C, Romero-Parra J, Simirgiotis MJ, Echeverría J. Chemical fingerprinting and biological evaluation of the endemic Chilean fruit *Greigia sphacelata* (Ruiz and Pav.) Regel (Bromeliaceae) by UHPLC-PDA-orbitrap-mass spectrometry. *Molecules*. 2020;25(16):3750.
59. Magaji UF, Sacan O, Yanardag R. Alpha amylase, alpha glucosidase and glycation inhibitory activity of *Moringa oleifera* extracts. *S Afr J Bot*. 2020;128:225–30. <https://doi.org/10.1016/j.sajb.2019.11.024>.
60. Jin W, Stehbens SJ, Barnard RT, Blaskovich MAT, Ziora ZM. Dysregulation of tyrosinase activity: a potential link between skin disorders and neurodegeneration. *J Pharm Pharmacol*. 2024;76(1):13–22.
61. Salah Maamoun H, Rabie GH, Shaker I, El-Sayed BAA. Biochemical properties of tyrosinase from *aspergillus terreus* and *Penicillium copticola*; undecanoic acid from *Aspergillus Flavus*, an endophyte of *Moringa oleifera*, is a novel potent tyrosinase inhibitor. *Molecules*. 2021;26(5). <https://doi.org/10.3390/molecules26051309>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

BioMed Central publishes under the Creative Commons Attribution License (CCAL). Under the CCAL, authors retain copyright to the article but users are allowed to download, reprint, distribute and /or copy articles in BioMed Central journals, as long as the original work is properly cited.