The Journal of Phytopharmacology 2026; 15(1):108-116
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2026; 15(1): 108-116
January- February
Received: 19-11-2025
Accepted: 24-03-2026
Published: 30-03-2026

doi: 10.31254/phyto.2026.15115

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Analysis of phytochemicals in leaf ethanolic extracts of *Moringa oleifera*

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ABSTRACT

Background: Known for its nutrient-rich content, the *Moringa oleifera* (MO) plant is prevalent in Africa and India. MO has significant anti-inflammatory, anticancer, and antimicrobial properties, making it a useful nutraceutical. **Objective:** To examine similarities and differences in fresh and aged MO leaf extracts phytochemical profiles. **Materials and Methods:** We used ethanolic plant leaf extracts from North American and Central American harvests (100% or 80% ethanol with 20% water) and analyzed total phenolic content, absorbance, and fluorescence profiles. We also performed capillary electrophoresis separations and collected liquid chromatography-mass spectrometry data. Furthermore, we examined mass spectral data from ethanolic extracts of both fresh and aged plant leaves. **Results:** Fresh aqueous ethanolic (80%) leaf extracts led to greater total phenolic content, greater absorbance and fluorescence signal, and more abundance in electropherograms by capillary electrophoresis with absorbance detection. Aged leaf extracts produced varied results. Thirteen phytochemicals were determined by LC-MS (isoamyl nitrite, ethosuximide M5, trolamine, octopamine (p-hydroxyphenylethanolamine), veratric acid, 6-methoxy-2-naphthylacetic acid, racepinephrine, pyridoxine, chlorogenic acid, o-coumaric acid, tiagabine, quercitrin, and dihydroquercetin) to be present in fresh and aged leaf extracts. **Conclusion:** Fresh and aged MO leaf extracts showed distinct phytochemical profiles. Fresh samples exhibit more compounds with phenolic structures and anti-inflammatory properties. Differences in these samples likely result from autooxidation and/or changes in environmental factors affecting plant growth.

Keywords: Capillary Electrophoresis, Nutraceuticals, Phenolic Content, Mass Spectral Data, Fresh and Aged Leaf Extractions.

INTRODUCTION

The tree species *M. oleifera* Lam. (MO) is found in Africa, the Middle East, and Asia [1]. MO is known as “the miracle tree” [2] because it has historically been used as a supplement, but its more popular uses have been in cuisine and cosmetics. Medicinally, MO has been used for hypertension, ulcers, bacterial infections, and inflammatory states [3]. While the bark, seed, leaf, and root of the plant provide natural product content, the plant leaf is preferred [3]. Sixty-six of the 168 compounds known MO compounds are bioactive [1,4]. Many of the bioactives are phenolic and exhibit anti-inflammatory, antimicrobial, antioxidant, anticancer, antiulcer, antidiabetic, and antitumor properties [3]. Given this range of health benefits, MO warrants additional study as a nutraceutical or supplement.

Phenols as Phytochemicals of Interest in MO extracts

Previously identified MO bioactive components are flavonoids and phenols [1]. Flavonoid compounds contain a phenol structural group with three aromatic rings. Traditionally, phenolic compounds have been part of plant defense systems. The oxidation process of bioactives in MO plant leaves as they age is not well understood. Browning that occurs in tea [5], wine [6], fruits [7,8], and vegetables [9,10] with age is an example of this process. The flavonoids quercetin, kaempferol, and catechin are known antioxidants present in MO. Quercetin possesses ROS-scavenging abilities and is not limited by structural following oxidation [11]. Glycosylated flavonoids are more prevalent in MO than other *Moringa* species [12]. Glycosylation allows the plant to adapt to environmental stressors, potentially increasing the abundance of evolutionary metabolites [12].

Flavonoid glycosylation also facilitates digestion in humans, increasing absorption and the likelihood of pharmaceutical use [12].

Differences in Growth Environment

Climates with tropical or subtropical characteristics support the largely drought-resistant MO species [13]. Recently, cultivation of MO has moved from Africa, the Middle East, and Asia [1, 13] to Central, North, and South America and Southeast Asia [4, 13]. Environmental growth conditions – geographical location, plant age, harvest season, soil pH, light intensity, and plant pruning – can affect antioxidant and phenolic content [14]. Niger, India, and Nicaragua MO plant leaves vary in antioxidant concentrations [14]. Nicaraguan MO leaves have the largest concentration of phenolic compounds [15]. UV light intensity increases concentrations of bioactive chemicals, a trend observed in many plant species [16], while elevated soil potassium and phosphorus levels increase phenolic and carotenoid concentrations [14]. Examining common bioactives present in leaf extracts from different geographical locations and storage conditions helps limit the effect of environmental growth conditions.

Motivation for extraction using biologically safe methods

Solvent and extraction method selection is crucial for the downstream usage of MO as a nutraceutical, as solvent removal is necessary. Prior MO leaf extractions have used methanol, ethanol, and water as solvents. Sultana *et al*. determined that shaken extraction preserved phenolic and flavonoid compounds better than heat degradation during refluxed extractions [17]. Maqsood *et al*. studied the effect of solvent polarity on the extraction process. Extracts from ethanol and methanol contained flavonoids, saponins, alkaloids, phenolics, and tannins [18]. With downstream purification and analysis, solvent removal is difficult, especially if animal model work is planned. Removal of ethanol and methanol is necessary, as they may cause toxicity in animal model studies [19,20].

In this work, extracts from fresh and aged samples of dried MO leaves from different geographical areas are analyzed for total phenolic content. Sample absorbance and fluorescence profiles, as well as capillary electrophoresis separations, are compared. In addition, common bioactive chemicals in fresh and aged MO samples are identified by LC-MS.

MATERIAL AND METHODS

Materials

Sodium tetraborate (CAS # 1330-43-4) was used to prepare a 10 mM borate buffer at pH=9.0. Ethanol, and sodium hydroxide (1 M) were reagent grade. MO leaf total phenolic content was determined by Folin-Ciocalteu's Phenol Reagent (Sigma F9252-100) with gallic acid (CAS # 149-91-7). Two samples of MO leaves were utilized. MO leaves were dried and blended into a powder (gift from Drs. Joshua Idassi and Jahangir Emrani at North Carolina A&T University, Greensboro, NC, North America, in 2016). These samples were utilized for capillary electrophoresis (fresh extraction) and aged samples (extracted after more than 5 years of storage). Fresh MO leaf samples collected from Nicaragua, Central America in 2022 (gift from Linda Gable, New Song Ministries) were used. All samples were stored at 4 °C in the dark before use.

***M. oleifera* Preparation and Extraction**

Powder samples (1.0 g) were ground to a uniform particle size. Extractions were performed at room temperature extractions in 100% ethanol or 80% ethanol/20% water with shaking for 4 h. Samples were filtered two times with 0.22 µm filter paper prior to storage at -25 °C.

Whole Leaf Extract Analysis

Total Phenolic Content

Folin Ciocalteu reagent was used to find the total phenolic content (TPC) of MO leaf extracts [21,22]. Standard solutions were prepared from 0, 1, 3, 5, 8, 11, and 15 µL of gallic acid stock solution diluted to 15 µL with 10 mM borate buffer. A volume of 15 µL of fresh or aged MO extract sample was added to 40 µL of DI water and 15 µL of Folin-Ciocalteu reagent. After a 5 min incubation at room temperature, reactions were quenched with 65 µL of 8% sodium carbonate. Following dilution to 200 µL the absorbance at 595 nm was measured using a BioTek Synergy H1 microplate reader. Sample total phenolic content was calculated in triplicate as a gallic acid equivalent using the equation $y = 0.0227x - 0.008$, $R^2 = 0.9998$.

Absorbance Spectroscopy

Fresh and aged MO leaf extracts (diluted 1:100 to 1 mL final volume in 10 mM borate buffer at pH 9) were examined by absorbance spectroscopy with a Thermo Scientific Nanodrop 2000 Spectrophotometer. Standard 1 cm glass cuvettes were washed twice with 95% ethanol, and a 10 mM borate buffer (pH 9) was used as a blank.

Fluorescence spectroscopy

Fresh and aged MO extracts (1:100 dilutions in 10 mM borate buffer pH 9) were examined by fluorescence spectroscopy with a Horiba Jobin Yvon FluoroMax-4. Excitation spectra were obtained at an emission wavelength of 460 nm using 5 nm excitation and emission slit widths. Emission spectra were obtained with an excitation wavelength of 355 nm using 6 nm excitation and emission slit widths.

Capillary electrophoresis

MO leaf extract separation took place with a Beckman Coulter P/ACE MDQ Capillary Electrophoresis System using a 50 µm x 59.8 cm capillary at 25 °C. Initially, the capillary was rinsed for 3 min with 1 M NaOH, DI water, and 10 mM borate buffer (pH = 9.0) at 40 psi, then the sample was injected at 1.0 psi for 5.0 sec and separated on a running borate buffer at 20.0 kV for 12.00 min. Detection was at 340 nm. Prior capillary electrophoresis protocols were used as the basis for this experiment [23-26].

Liquid Chromatography -Mass Spectrometry

An Agilent LC-MS QTOF 6540 with an Agilent 1260 HPLC, HiP Sampler, binary pump, column comp, and Dual Agilent Jet-Stream Electrospray Ionization (ASJ-ESI) was used for LC-MS detection. For HPLC separation, a mobile phase consisting of 0.1% formic acid (v/v) in water (solvent A) and 0.1% formic acid (v/v) in acetonitrile (solvent B) was used at 0.8 mL/min, a column temperature of 30 °C, and a maximum pressure of 600.0 bar on a C18 column. Injection volumes were 1.00 µL. Elution conditions were: 0.0–2.0 min 2% solvent B. 2.0 -12.0 min 2-100% solvent B, 12.0-15.0 min 100% solvent B, 15.1–18.0 min 100-2% solvent B. Eluent peaks were monitored by positive ion mode ESI, from *m/z* 110 to 1700 Da, with gas temperature at 250 ºC, flow of 8 L/min, 35 psi nebulizer pressure, sheath gas temperature at 300 ºC and sheath gas flow at 11 mL/min, nozzle voltage of 500 V, and capillary voltage of 3500 V. Reference masses of 121.050 and 922.009 Da were used. Mass Profiler Professional software in the Mass Hunter Workstation was used to analyze spectra with the Metlin spectral library.

RESULTS

Total Phenolic Content

The total phenolic content (TPC) method was used to determine the amount of phenols in MO leaf extracts. TPC was calculated with triplicate samples using a calibration curve as described in the Methods. Table 1 gives the TPC amount for fresh and aged MO samples. TPC values were highest for the 80% ethanol/20% water extraction of fresh samples.

Optical Spectroscopy

Absorbance spectra of the MO extracts show absorbance peaks at 270 nm and 360 nm for 80% ethanol/20% water extracts. Figure 1 shows the absorbance of chromophores in the 100% ethanol extracts is red-shifted and less intense. Fluorescence intensity in bioactive emission areas was present in both 100% ethanol and 80% ethanol / 20% water MO leaf extracts, as indicated in Figure 2. Spectra were most intense for aqueous-ethanolic extractions. Aged ethanolic samples also appeared more intense than fresh ethanolic samples in this spectral region.

Capillary Electrophoresis

Separation of chromophores in freshly extracted MO leaves was monitored by capillary electrophoresis, as shown in Figure 3. As in the optical spectroscopy analysis, the aqueous ethanolic extract shows a higher abundance of compounds that interact with light in the electropherogram.

LC-MS

The LC-MS identified compounds present in fresh and aged MO leaf extracts in 100% ethanol solvent are listed in Tables 2 and 3. Eighty-one compounds were present in the fresh leaf samples, while 75 were present in the aged samples. The common compounds in both samples were isoamyl nitrite, ethosuximide M5, trolamine, oxtopamine (p-hydroxyphenylethanolamine), veratric acid, 6-methoxy-2-naphthlacetic acid, racepinephrine, pyridoxine (Vitamin B6), chlorogenic acid, o-coumaric acid, tiagabine, quercitrin, and dihydroquercetin as listed in Table 4.

Table 1: Fresh and Aged MO samples Total Phenolic Content in different ethanolic solvents

Sample	TPC (mM)
100% ethanol Fresh	30.37 ± 0.04
100% ethanol Aged	38.88 ± 0.04
80% ethanol / 20% water Fresh	216.27 ± 0.04
80% ethanol / 20% water Aged	140.21 ± 0.04

Table 2: LC-MS identified compounds in fresh 100% ethanolic MO leaf extracts with retention times (RT) and m/z values

Compound	RT	m/z	Identity
1	1.074	145.1573	spermidine
9	1.146	228.1704	10-keto tridecanoic acid
12	1.245	314.1825	pergolide
14	1.285	146.0686	beta-ureidoisobutyric acid
15	1.288	147.0527	o-acetylserine
18	1.300	225.1232	4'- hydroxyminoxidil
21	1.314	143.07	cyclocreatine
23	1.391	133.0735	1,4-dideoxy-1,4- imino-D-arabinitol
24	1.449	140.0582	1,4- methylimidazoleacetic acid
26	1.466	117.0786	isoamyl nitrite
27	1.472	145.1096	2R-aminoheptanoic acid
33	1.527	155.0578	ethosuximide M5
36	1.557	145.0847	4- (diaminomethylideneamino) butanoic acid
39	1.570	153.0646	4,6-diamino-5- formamidopyrimidine
40	1.579	215.0815	kinetin
42	1.583	309.1057	N-acetyl-a- neuraminic acid
49	1.710	210.037	mucic acid
52	1.728	141.0421	2- aminomuconate 6- semialdehyde
54	1.736	146.021	2-ketoglutaric acid
61	1.872	149.1047	trolamine
72	2.140	182.0573	veratric acid
73	2.140	153.0785	octopamine (p- hydroxyphenylethanolamine)
75	2.169	178.0625	10-hydroxy-8E- decene-4,6-diynoic acid
76	2.173	216.0782	6-methoxy-2- naphthylacetic acid
79	2.177	270.1102	idebenone metabolite
80	2.183	166.0626	atrolactic acid
81	2.191	208.073	benzylsuccinic acid
84	2.214	150.0676	4-ethylbenzoic acid

91	2.259	216.0781	euparin
96	2.760	161.0683	L-2-aminoadipic acid
100	2.841	267.096	neuraminic acid
101	3.196	136.0379	hypoxanthine
104	3.256	144.0999	4-guanidinobutanamide
106	3.274	183.089	racepinephrine
118	3.560	309.1208	tranylcypromine glucuronide
119	4.476	129.0787	vigabatrin
120	4.508	378.2031	18-hydroxycortilsol
122	4.819	362.1715	thyrotropin releasing hormone
124	4.903	205.0736	deschlorobenzoyl indomethacin
125	4.904	222.1003	4-aminohippurate
126	4.904	163.063	p-acetaminobenzaldehyde
127	4.936	169.0735	pyridoxine
128	4.962	354.0951	chlorogenic acid
129	4.962	180.042	caffeic acid
131	5.167	544.2662	7- deacetylkhivorin
134	5.309	164.047	o-coumaric acid
136	5.427	234.1005	p-hydroxyprimidone
137	5.428	161.0838	indole-3-ethanol
138	5.671	198.054	syringic acid
139	6.200	432.106	cosmosiin
140	6.226	610.1543	rutin
141	6.313	432.1061	1-methyl-4-nitro- 5-(S-gluctathionyl) imidazole
142	6.370	221.0574	histidinol phosphate
143	6.371	320/0533	dihydromyricetin
144	6.487	264.0576	enoximone sulfoxide
145	6.590	196.1097	4-(2- hydroxypropoxy)-3,5- dimethyl-phenol
146	6.597	275.0479	phenylacetic mustard
147	6.615	304.0581	dihydroquercetin
148	6.615	448.1001	quercitrin
149	6.778	267.05	1-alkenyl-2-acyl- glycerophosphoethanolamine
156	7.388	375.1349	tiagabine
157	7.686	329.2172	8-hydroxyamoxapine
160	8.480	273.2662	C16 sphinganine
161	8.490	506.3954	(20S)-24- hydroxy-19- norgeminivitamin D3
162	8.511	596.3904	26-o-[beta-d- glucopyranosyl]-25R- furostan 3beta,22alpha,26- triol
164	8.611	290.1877	8-hydroxy-17- octadecene-9,11-diynoic acid
165	8.666	256.1036	dinorpromazine
166	8.671	300.0674	sulfaquinoxaline
167	8.694	371.1031	nedocromil
168	9.046	297.2666	(Z)-N-(2- hydroxyethyl)hexadec-7- enamide
171	9.803	422.2662	lovastatin acid (mevinolinic acid)
172	9.858	294.2192	alpha-9(10)- EpODE
175	10.115	268.1494	equilin
176	10.487	594.3731	7,8-didehydroastaxanthin
178	10.814	381.2875	5,6-DiHETrE-EA
179	10.860	321.2663	anandamide (18:3, n-3)
180	11.335	322.2504	12R-HETrE

181	11.415	341.2926	N-acetylesphingosine
183	10.098	512.2812	3-deoxo-3beta- acetoxydeoxydihydrogedunin
184	14.045	558.3229	cucurbitacin B
185	16.604	141.0898	histidinol

Table 3: LC-MS identified compounds in aged 100% ethanolic MO leaf extracts with retention times (RT) and m/z values

Compound	RT	m/z	Identity
1	1.076	145.1577	spermidine
9	1.169	131.1289	agmatine
11	1.283	147.0529	o-acetylserine
17	1.391	133.0737	1,4-dideoxy-1,4- imino-D-arabinitol
19	1.406	195.1105	meglumine
22	1.459	117.0788	isoamyl nitrite
23	1.471	145.1101	2R-aminoheptanoic acid
24	1.472	159.0893	2- methylbutyrylglycine
28	1.506	161.105	bis (2- hydroxypropyl) amine
30	1.507	209.1263	minoxidil
33	1.526	155.0581	ethosuximide M5
36	1.564	241.1175	sapropterin
37	1.572	179.0804	glucosamine
39	1.575	153.0647	4,6-diamino-5- formamidopyrimidine
48	1.782	205.1313	dexpanthenol
52	1.837	140.0583	1,4- methylimidazoleacetic acid
54	1.891	173.0685	2,6- piperidinedicarboxylic acid
55	1.895	149.105	trolamine
68	2.153	153.0788	octopamine (p- hydroxyphenylethanolamine)
69	2.154	182.0577	veratric acid
71	2.178	225.1	L-4-hydroxy-3- methoxy-a- methylphenylalanine
74	2.190	216.0786	6-methoxy-2- naphthylacetic acid
75	2.195	166.0627	atrolactic acid
76	2.197	178.0627	8S-hydroxy-2- decene-4,6-diynoic acid
78	2.197	150.0678	4-ethylbenzoic acid
79	2.199	270.1103	idebenone metabolite
89	2.791	267.0968	vidarabine
98	3.292	183.0893	racepinephrine
108	3.671	181.1101	2-hepten-4-yn-1- amine, N,6,6-trimethyl-, (E)- metabolite
109	3.673	227.1157	hydroxyphenoxyethylaminohydroxypropanol
116	4.475	188.0682	ethyl oxalacetate
117	4.476	128.0471	6-hydroxy-2-hexynoic acid
118	4.476	146.0576	alpha- ketopantoic acid
123	4.730	399.1894	diethylpropion
124	4.792	253.1313	4-(2-hydroxy-3- isopropyl- aminopropyl)benzoic acid
126	4.901	222.1003	4- aminohippurate
127	4.901	205.0735	deschlorobenzoyl indomethacin
128	4.901	163.0629	p-acetaminobenzaldehyde
129	4.930	169.0753	pyridoxine (Vitamin B6)
131	4.958	354.095	chlorogenic acid
132	4.598	180.0419	m-hydroxyphenylpyruvic acid
139	5.175	148.0521	p-hydroxycinnamaldehyde

140	5.176	176.0471	10-hydroxy-8E- decene-2,4,6-triynoicacid
143	5.176	188.0834	5,8,11- dodecatriynoic acid
145	5.258	170.1304	2- decylenic acid
149	5.305	164.047	o-coumaric acid
151	5.499	424.1346	ginkgolide B
154	5.907	434.1764	lonchocarpic acid
156	6.195	432.106	1-Methyl-4-nitro- 5-(S-Gluctathionyl) Imidazole
158	6.328	236.1048	carboxyibuprofen
159	6.368	302.0427	morin
160	6.368	464.0958	demeclocycline
164	6.368	464.0958	benzenemethanol, 2-(2- aminopropoxy)-3-methyl-
165	6.610	448.1007	quercitrin
166	6.611	304.0583	dihydroquercetin
173	6.893	140.1198	2-nonenal
182	7.386	375.1353	tiagabine
183	7.681	584.2818	ouabain
186	8.273	453.3091	10- deoxymethymycin
188	8.351	417.1495	mefenamic acid metabolite
190	8.468	528.3777	isorenieratene (leprotene)
198	8.676	290.1881	8-hydroxy-17- octadecene-10,12-dinoic acid
200	8.680	348.1911	sodium chlorovulone III
203	8.997	324.1936	12-oxo-14,18- dihydroxy-9Z,13E,15Z- octadecatrienoic acid
204	8.999	306.183	tetranor iloprost
209	9.434	390.2379	gitoxigenin
210	9.859	294.2195	12-oxo-9- octadecynoic acid
212	9.990	336.23	11-deoxy-PGE2
213	9.993	376.2226	resolvin D2
215	10.052	318.2195	5S-HEPE
217	10.413	352.2249	thromboxane A2
220	10.485	576.3629	1- hydroxyvitamin D3 3-D- glucopyranoside
222	10.642	360.2275	pregn-4-ene- 3,20-dione, 6a,17- dihydroxy-6-methyl-
225	11.334	322.2509	12R-HETrE
226	16.604	141.0899	histidinol

Table 4: Common compounds with a known health benefit identified from LC-MS of ethanolic fresh and aged MO samples from Central and North America

Compound	Known Applications
isoamyl nitrite	Anti-hypertensive [29], Vasodilator [30]
ethosuximide M5	Anti-convulsant [31]
trolamine	Osteoarthritis [32], Anti-inflammatory [33] Reduces radiation-induced skin damage [33]
octopamine (p- hydroxyphenylethanolamine)	Hypotension [34]
veratric acid	Anti-inflammatory [35], Antioxidant [35]
6-methoxy-2- naphthylacetic acid	Non-steroidal anti-inflammatory [36]
racepinephrine	Bronchodilator [37]
pyridoxine (Vitamin B6)	Nausea/ vomiting medication during pregnancy [38]
chlorogenic acid	Antioxidant [39, 40], Cardioprotective [40]
o-coumaric acid	Antioxidant [41], Anti-obesity [42], Anti-carcinogenic [43]
quercitrin	Antioxidant [44], Anti-inflammatory [44]
dihydroquercetin	Antioxidant [45], Anti-carcinogenic [45] Anti-tumor [45], Anti-cardiovascular [45]
tiagabine	Anti-convulsant [46]

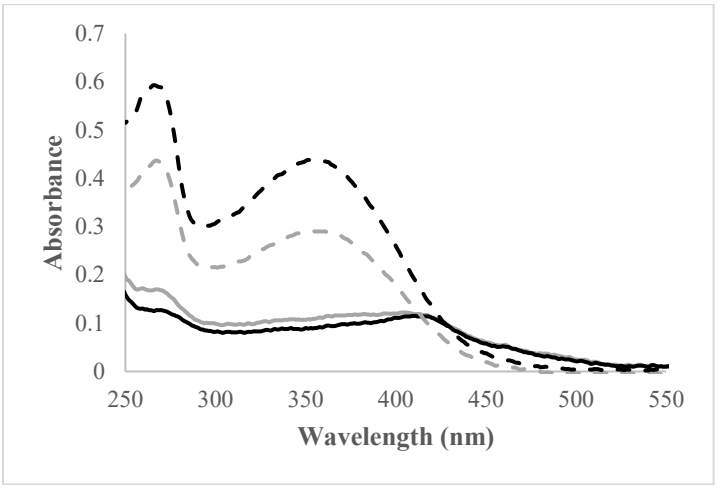

Figure 1: Absorbance spectroscopy of 1:100 diluted MO samples in ethanol (solid) and 80% ethanol/ 20% water extractions (dashed). Fresh sample extracts are black, and aged sample extracts are gray

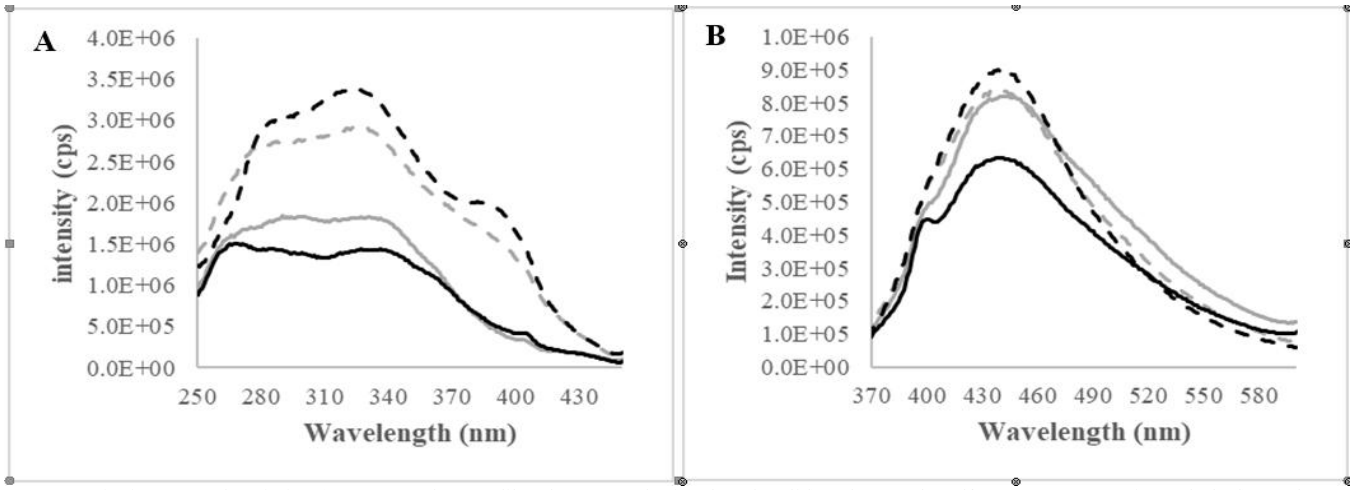

Figure 2: Excitation (A) and Emission (B) spectra of 1:100 diluted MO samples in ethanol (solid) and 80% ethanol/ 20% water extractions (dashed). Fresh sample extracts are black, and aged sample extracts are gray

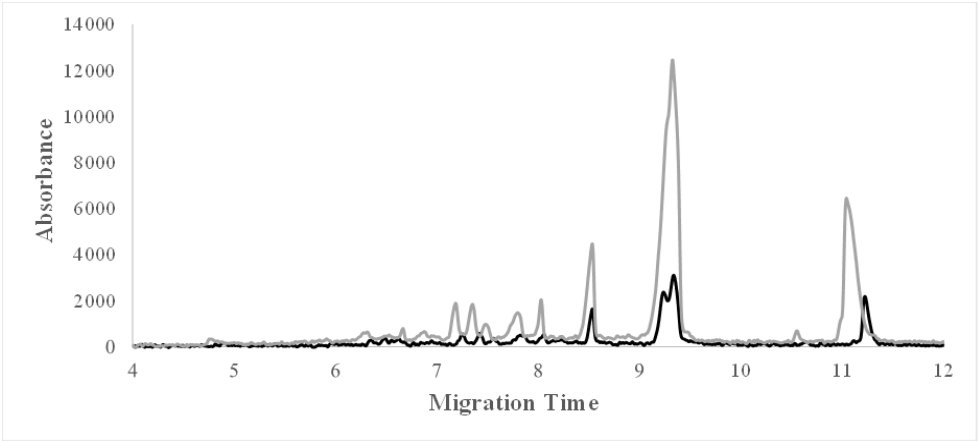

Figure 3: Electropherograms absorbance detection at 340 nm of MO leaf samples extracted in 100% ethanol (black) and 80% ethanol/ 20% water (gray)

DISCUSSION

This analysis offers new insights into MO leaf extractions at two ethanol concentrations, using fresh and aged samples from Central and North America. TPC values were highest for the 80% ethanol/20% water extraction method in fresh samples. This indicates that phenolics may degrade over time in aged ethanol-water-extracted samples, possibly through autoxidation. This trend reversed in the 100% ethanol MO leaf extractions. Alcohol groups on phenol ring structures increase molecule polarity, which explains why aqueous extracts (which have higher polarity than alcohol extracts) are the preferred method for MO bioactive leaf extractions. Phenols exhibit absorbance at 270 nm and 360 nm in the UV-A and UV-B regions, indicating high phenolic and flavonoid concentrations in the 80% ethanol/20% water extracts [27]. Differences in absorbance spectra peaks are more significantly affected by solvent polarity than by the sample age. Excitation spectra replicate the absorbance spectra, revealing more molecules capable of fluorescence in the 80%

ethanol/20% water extracts. As expected, the fluorescence emission curve for the extract was most prominent in the 400-530 nm range, known for phenol and some flavonoid fluorescence [27, 28].

Capillary zone electrophoresis illustrated separations with 340 nm absorbance detection of MO leaf extracts. The 80% ethanol/20% water extraction yields sharper peaks. This method may be further exploited by using standard addition of a phenol or flavonoid present to standardize the extraction process. [23-28].

The aging of MO leaf phytochemical content has not been studied previously. Aged MO leaf extracts have fewer phytochemicals than fresh MO leaf extracts as detected by LC-MS in Tables 2 and 3. However, it may be useful to focus on compounds that appear independent of sample age or growth conditions when using MO as a nutraceutical. Table 4 gives 13 compounds present in both fresh North American and aged Central American MO ethanolic samples. Antioxidants, anti-inflammatory agents, and anticarcinogenic substances are the most common chemicals to investigate further, as they are found in extracts regardless of these factors.

CONCLUSION

Fresh and aged MO leaf extracts were analyzed to assess variation in phytochemical content. TPC shows phenols in fresh and aged samples, with higher TPC values in fresh MO leaf extracts. This trend continues with our optical spectroscopy and capillary electrophoresis analysis. The extraction solvent significantly influenced both phenolic and flavonoid content. Both fresh and aged MO leaf extracts contain anti-inflammatory compounds. Molecules with anticarcinogenic and antioxidant effects were also present, as determined by LC-MS. The phytochemical abundance in fresh and aged MO extracts indicates much promise for the plant as a nutraceutical. Still, more work is needed to identify chemicals that are independent of leaf aging and/or environmental growth conditions.

Acknowledgements

We thank the University of Southern Florida Chemical Purification Analysis and Screening Core Facility for assistance with mass spectrometry and Dr. Libby Puckett at Appalachian State University for help with capillary electrophoresis. MO samples were gifts from Linda Gable (New Song Ministries) and Drs. Joshua Idassi and Jahangir Emrani (North Carolina A & T University). We also thank the Appalachian State University Honors College. The Office of Student Research at Appalachian State University and the George Williams Garrett Memorial Scholarship supported this project financially.

Conflict of interest

The authors declared no conflict of interest.

Financial Support

None declared.

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HOW TO CITE THIS ARTICLE
Bennett AE, Urbie-Rheinbolt F, Sciara T, Mowa CN, Cecile JP. Analysis of phytochemicals in leaf ethanolic extracts of *M. oleifera*. J Phytopharmacol 2026; 15(1):108-116. doi: 10.31254/phyto.2026.15115