Chemical and biological studies on *Moringa oleifera* L. cultivated in Egypt

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Background
Nowadays, *Moringa oleifera* is being cultivated in Egypt on a wide scale owing to its proven medicinal and economic benefits.
Objective
The aim was to estimate quantitatively different phenolic acids and flavonoids in the *M. oleifera* L. leaves. Moreover, gas chromatography–mass spectrometry (GC/MS) analysis was carried out to evaluate seed oil obtained by three different methods of extraction. Different plant extracts were tested for their hepatoprotective, anticancer, and antibacterial activities.
Materials and methods
Quantification of different phenolic acids and flavonoids in *M. oleifera* L. leaves was carried out using HPLC. GC/MS was used to determine fatty acid methyl esters of *M. oleifera* L. seed oil, extracted by three different methods (cold press, solvent extraction, and ultrasound-assisted extraction). Moreover, in-vitro investigations of hepatoprotective, anticancer, and antibacterial activities were done.
Results and conclusion
HPLC profiling of leaves extract indicated that ellagic acid is the major phenolic acid (120.15 mg/g). Quercetin and rutin were recorded as major flavonoids. GC/MS of seed oil extracted by ultrasound-assisted extraction showed the presence of higher content of oleic acid comparable with other extraction techniques. The petroleum ether fraction of the leaves showed the most potent hepatoprotective and anticancer effects, whereas the ethanolic extract was active against the tested gram-positive and gram-negative microorganisms. Our findings confirm that *M. oleifera* L. cultivated in Egypt has unique phytochemical content (comparable with *M. oleifera* cultivated in other countries); consecutively, it has many potent biological activities. So, it is highly recommended to cultivate the plant species on a wide scale to make use of its constituents in pharmaceutical and nutraceutical industries.

Keywords:
antibacterial, anticancer, flavonoids, phenolic acids, ellagic acid, hepatoprotective, HPLC, *Moringa oleifera*, seed oil, ultrasonic

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Introduction

Moringa oleifera Lam. (Family: Moringaceae) commonly known as Drumstick tree has an impressive range of medicinal and nutritional uses. Different parts of the plant contain a profile of various important minerals and are a good source of proteins; vitamins A, C, and E; β-carotene; amino acids; and various polyphenolics. The extracts of the leaves, seeds, and roots of *M. oleifera* have been extensively studied for many potential uses, including wound healing, antitumor, antifertility, hypotensive, analgesic, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, hypocholesterolemic, antifungal, antibacterial, and antioxidant activities [1]. *M. oleifera* is widely used in different countries in the traditional medicine, for example, in Malaysia, a poultice of young leaves is applied to the abdomen to expel tapeworms. Pods and flowers have also been used as anthelmintics. The leaves have been used in India as an antihypertensive; diuretic; antidiabetic; antianxiety agent; antidiarrheal; and to treat dysentery, colitis, and gonorrhea. Poultices made from the leaves have been used to treat glandular inflammation, headache, bronchitis, ear and eye infections, scurvy, and cataracts. In Nicaragua, India, Guatemala, and Senegal, poultices of young leaves are used to treat skin infections. Pods have been used to treat hepatitis and relieve joint pain. The roots of *M. oleifera* have been used in India as a laxative; to treat edema; to clean wounds and ulcers; to treat hiccups; to relieve pain associated with toothache and earache; and to treat asthma, gout, rheumatism, kidney stones, and diseases of the liver and spleen. In addition, *M.*

oleifera has been used to arouse patients from comas and stupor. The bark of roots and stems has been used to treat wounds and skin infections, scurvy, sore eyes, delirium, digestive complaints, and to prevent snake venom from disseminating in the bloodstream. In addition, the bark has been used as an aphrodisiac and abortifacient and to treat upper respiratory tract infections. *Moringa* gum has been used to relieve pain from headache and gastroenteritis. Natives of India and Senegal have used the gum to treat fever, dysentery, asthma, dental caries, syphilis, and rheumatism, as well as to induce abortion. Seeds have also been used to treat fever, tumors, warts, hysteria, scurvy, prostate, and bladder problems. In addition, seeds act as a tonic and purgative [2].

The oil content of the plant seeds ranges from 35 to 41% and is a rich source of omega-9 fatty acids, for example, oleic acid; however, the seeds are underutilized as an oil source [3]. In this study, a quantitative estimation of different phenolic acids and flavonoids was done. In addition, different plant extracts were tested for their hepatoprotective, anticancer, and antibacterial activities.

Materials and methods

Plant material

Moringa oleifera L. leaves and seeds were picked from trees grown on sandy soil in El-Sharkia Governorate, Egypt, in June 2014. The plant was identified by Professor Dr Aboelfetoh Mohamed Abd-Alla, Egyptian Scientific Society of Moringa, National Research Centre, Giza, Egypt. The different plant parts were air-dried, ground into a fine powder, and kept for investigation.

Solvents and chemicals

Distilled water, absolute ethyl alcohol, hexane, methanol, petroleum ether, methylene chloride, ethyl acetate, butanol, HPLC grade methanol, tri-fluoroacetic acid, acetonitrile, and 3-[4,5-dimethylthiazol-2-yl]-2,5 dipheny tetrazolium bromide (MTT) were obtained from Sigma (St Louis, Missouri, USA).

Microorganisms

Ten bacterial strains were used in the study; among these were seven gram-negative bacteria, namely, *Escherichia coli*, *Coliform* spp., *Klebsiella* spp., *Tannerella forsythia*, *Prevotella intermedia*, *Pseudomonas* spp., and *Aggregatibacter actinomycetemcomitans* and three gram-positive bacteria, namely, *Staphylococcus aureus*, *Bacillus cereus*, and *Streptococcus-B-hemolytic.*

Quantification of phenolic acids and flavonoids using HPLC

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using the Eclipse Plus C18 column (4.6×250 mm, i.d. 5 μm). The mobile phase consisted of water (A) and 0.02% tri-floro-acetic acid in acetonitrile (B) at a flow rate of 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A), 0–5 min (80% A), 5–8 min (40% A), 8–12 min (50% A), 12–14 min (80% A), and 14–16 min (80% A). The multiwavelength detector was monitored at 280 nm. Injection volume was 10 μl. The column temperature was maintained at 35°C.

Ultrasonic processor (UP) (400 W, 24 kHz, Hielscher)

The system with an ultrasonic probe with a tip diameter of 20 mm (the horn tip position inside the extraction vessel was 1 cm under the solvent level) was used.

Extraction and fractionation

Extraction of plant leaves

Overall, 100 g of the powdered leaves of *M. oleifera* L. was extracted till exhaustion with 80% methanol, then filtered and evaporated under reduced pressure at 45°C to give 23 g (23%). The residue was suspended in distilled water and then the residual aqueous solution was left in the refrigerator overnight and filtered. Then, it was successively extracted with petroleum ether, methylene chloride, ethyl acetate, and butanol (3×200 ml each). The obtained fractions were evaporated under reduced pressure to obtain 23, 1.06, 0.94, 1.48, and 16.03 g, respectively. Another batch (100 g) of the powdered leaves was extracted till exhaustion with distilled water, filtered, and evaporated under reduced pressure to give about 17 g. Moreover, absolute ethanol extract of the leaves was prepared and kept for antibacterial evaluation.

Preparation of seed oil

The seed oil was prepared by three different methods as follows:

Solvent extraction (traditional method)

M. oleifera seed powder was prepared by grinding 10 g of the seeds in a clean coffee grinder for 30 s. The seed powder was soaked in 100 ml of n-hexane at room temperature for 72 h and filtered. The residue obtained was again extracted twice with 100 ml of n-hexane. The filtrates were collected and concentrated by using a rotary evaporator under vacuum at 40°C. The obtained oil was weighed (3.67 g) and stored at 4°C before analysis.

Ultrasound-assisted extraction

The device was operated at a frequency of 24 KHz and an ultrasonic input power of 400 W. Overall, 10 g of seed powder was mixed with 100 ml of n-hexane. Upon completion of extraction, the extract was removed from the vessel, filtered, and evaporated under vacuum. The extraction yield was 33.1 g and kept at 4°C before analysis.

Cold press extraction

M. oleifera mature seeds were dried in sun for 2–3 days. The dried seeds were sent to shell removing to unit, to get the kernels. The kernels were further dried for a week at the room temperature and handled carefully for cleanliness. Overall, 10 g of the seed was poured in the receiving funnel of the cold presser. After ten minutes, the oil was dripping from the outlet and collected in a container that was previously weighed. Overall, 2.53 g was obtained and kept at 4°C before analysis.

Table 1 summarizes the extraction conditions and the oil yield for each experiment.

Samples of the oil obtained by seed hexane extraction, oil obtained by seed compression, total 80% methanolic, aqueous extracts, petroleum ether, methylene chloride, ethyl acetate, and butanol fractions (M1–M8 fractions, respectively) of *M. oleifera* L. leaves were investigated for their hepatoprotective and cytotoxic activities.

Preparation of FAMEs

One ml of each oil sample was subjected to the following methylation method: 2 ml N/2 alcoholic KOH was added to each sample in a test tube. The fatty acid methyl esters (FAMEs) were extracted by shaking with 4-ml hexane for 30 s. The hexane layer was separated and washed with distilled water, dehydrated over anhydrous sodium sulfate, and then subjected to gas chromatography–mass spectrometry (GC/MS) analysis.

In-vitro bioassay on human hepatocellular carcinoma (hepG2) cells

Cell propagation and maintenance

Human hepatocellular carcinoma (HepG2) cells were propagated in the proper conditions (at 37°C and 5% CO_2), maintained in RPMI-1640 with 1% l-glutamine, and supplemented with 10% fetal calf serum for growth and 1% penicillin/streptomycin. When the cells are ~80% confluent, they were subcultured using Trypsin-EDTA protocol [4]. The second round of HepG2 cells sub-culturing was used for 96-well plates seeding, and after that, drug screening was done for testing the hepatoprotective and anticancer activities.

Table 1 Seed oil extraction by three different methods

Method of extraction	Cold compression	Traditional solvent extraction	Ultrasonic assisted extraction
Solvent used	–	Hexane (3×100 ml)	Hexane (100 ml)
Time	10 min	3 days	90 min
Yield percentage (%)	25.3	36.7	33.1

Hepatoprotective effect of Moringa oleifera L. samples

Carbon tetrachloride (CCl_4)-induced hepatocytes injury was carried out according to Raj *et al*. [5]. The screening of hepatoprotective activity was based on the protection of human liver-derived HepG2 cells against CCl_4-induced damage [6] determined by estimating mitochondrial synthesis using the tetrazolium assay [7]. Concentrations of the tested samples in the range of 0, 20, 40, 60, 80, and 100 μg/ml and standard drug Silymarin at 250 μg/ml which was proved as safe hepatoprotective dose of the positive reference (silymarin) were investigated (note: above this concentration, the extracts were toxic to the cells).

Cytotoxic effect of Moringa oleifera L. samples

The tetrazolium assay is based on the conversion of MTT into formazan crystals by living cells, determining mitochondrial activity [8]. The potential cytotoxic effects of the studied samples (M1–M8) on the liver cancer HepG2 cell line in concentrations of 0, 20, 40, 60, 80, and 100 μg/ml were estimated by MTT assay. The cytotoxic activity (%) of the *M. oleifera* L. samples fractions was calculated and compared with the control.

Statistical analysis

The results were presented as the mean of three independent experiments and the SD. The IC_{50} was obtained using the four-parameter nonlinear regression on GraphPad prism.

The antibacterial assay

Fresh leaves' water extract and the 100% ethanol extract were tested for their antibacterial activity. In-vitro antibacterial test was then carried out by disc diffusion method [9].

The discs were impregnated with 10 μl of the three different samples. Negative controls were prepared using the same solvents to dissolve the plant extracts. Imipenem (10 μg disc-1) was used as a positive control to determine the sensitivity of bacterial strain. Antimicrobial activity was evaluated

by measuring the zones of inhibition against the tested bacteria. Each assay was carried out in triplicate.

Minimum inhibitory concentration)

Minimum inhibitory concentration of three different samples, as mentioned earlier, was determined by 0.1 ml of samples added into the test tubes separately, containing 9 ml of a standardized suspension of tested bacteria.

The test tubes were incubated at 37°C for 24 h. Controls were used with the test organisms, using distilled water instead of the plant extract. The least concentration of the samples with no visible growth was taken as the minimum inhibitory concentration.

Results and discussions

Identification and quantification of different phenolic acids and flavonoids

Identification of phenolic acids and flavonoids from leaf extracts of *M. oleifera* L. was analyzed by HPLC, and obtained data of the retention time and concentrations are presented in Table 2.

The obtained results showed that the butanol fraction contains the highest amounts of both gallic and chlorogenic acids (15.42 and 12.75 mg/g, respectively), whereas the ethyl acetate fraction contained the high concentrations of syringic and ellagic acids (9.28 and 120.15 mg/g, respectively). The flavanone, naringenin was detected in both ethyl acetate and butanol fractions (21.56 and 15.95 mg/g, respectively), which exceeds its content in its most abundant sources [10]. The HPLC analysis showed that *M. oleifera* L. cultivated in Egypt could be considered as a great source of phenolic acids and flavonoids comparable with *M. oleifera* cultivated in other countries, as shown in Table 3. The present study showed that phenolics and flavonoids, especially ellagic acid, were present in a very high amount (120.15 mg/g) compared with the *M. oleifera* species cultivated in other countries. To the best of our knowledge, the highest reported concentration of ellagic acid was reported in the *M. oleifera* L. cultivated in Nigeria (52.95 mg/g) [14], whereas it is almost in double in that cultivated in Egypt.

Among the identified compounds, different phenolic acids and flavonoids with numerous biological activities were detected. These different compounds possess different biological effects, such as antioxidant activity (which may play a role in hepatoprotection activity) which was exerted by compounds such as gallic [15], chlorogenic [16], ellagic acids [17], rutin [18], and quercetin. Moreover, powerful anticancer activity was reported for chlorogenic [16], syringic [19], ellagic acids [17], naringenin [20], and quercetin. In addition, ellagic acid and naringenin [21] were reported to exert antimicrobial activity.

These results come in accordance with our biological findings which support that different plant extracts and fractions possess potent hepatoprotective, cytotoxic, and antibacterial activities.

GC/MS analysis of FAMEs of seed oil

The data of GC/MS analysis of FAME samples are compiled in Table 4. The previous results revealed that the seed oil obtained by ultrasound-assisted extraction (UAE) has palmitic acid (saturated fatty acid) and oleic acid (unsaturated fatty acid) as the major constituents in a percentage of 14.77 and 64.78, respectively. Both oleic and palmitic acids have many potent biological activities. This unique chemical composition of seed oil was only obtained by the UAE, as the oil obtained by cold compression has only palmitic acid as its major constituent in a percentage of 15.73%, whereas oleic acid was not detected and the seed oil obtained by solvent extraction has 13-Octadecenoic acid as its major constituent in a percentage of 51.37. However, both oleic and palmitic acid were absent, which give high curative and nutritive value for *M. oleifera* seed oil obtained by UAE technique. Oleic acid represents the principal constituent in seed oil obtained by the ultrasonic hexane extraction method (which is an innovative extraction technique). It presents in very high impressive percentage (64.78%), which gets close to the oleic acid content in olive oil (ranges from 56 to 84%), and the healthful properties of olive oil have been, until recently, exclusively attributed to its high monounsaturated fatty acid content, mostly in the form of oleic acid [22].

Different fatty acids were identified in *M. oleifera* L. seed oil with potent biological activities such as antioxidant activity, which was exerted by palmitic [23] and oleic acids [23,24], and also a hepatoprotective activity was reported for palmitoleic acid [25]. Moreover, oleic acid was found to have potent anticancer activity. These results emphasize our biological findings that the plant seed oil exerts hepatoprotective and cytotoxic activities.

So, further biological studies should be done to make optimum use of *M. oleifera* L. seed oil obtained, especially by ultrasonic technique owing to its exceptional properties.

Table 2 HPLC quantification of phenolic acids and flavonoids of different plant extracts and fractions

Standard name	Retention time (min)	80% MeOH extract (M3)		CH_2Cl_2 fraction (M6)		EtoAc fraction (M7)		n-BuOH fraction (M8)	
		Retention time (min)	Concentration (mg/g)	Retention time (min)	Concentration (mg/g)	Retention time (min)	Concentration (mg/g)	Retention time (min)	Concentration (mg/g)
Gallic acid	3.093	ND	–	3.093	1.94	3.095	5.02	3.098	15.42
Chlorogenic acid	3.419	–	–	3.370	1.01	3.497	2.58	3.479	12.75
Catechin	3.753	3.777	0.81	3.809	0.03	3.716	1.99	3.738	0.38
Caffeine	3.966	–	–	3.953	0.02	4.019	0.13	4.017	0.10
Methyl gallate	4.549	–	–	4.454	0.06	4.555	2.88	4.481	3.75
Coffeic acid	4.852	5.048	0.33	4.979	0.02	4.879	1.63	4.965	0.36
Syringic acid	5.217	5.452	0.39	5.314	6.84	5.409	9.28	5.379	0.73
Rutin	5.478	5.802	0.65	ND	–	5.631	3.17	5.661	10.34
Pyro catechol	5.733	–	–	5.708	0.04	ND	–	ND	–
Ellagic acid	6.634	–	–	6.818	0.64	6.899	120.15	ND	–
Coumaric acid	7.480	ND	–	7.460	0.02	7.609	0.57	ND	–
Vanillin	8.198	8.415	0.14	8.424	0.06	8.187	0.47	8.244	1.30
Ferulic acid	8.765	ND	–	8.777	0.13	9.075	0.05	ND	–
Naringenin	9.298	9.417	2.13	9.310	0.15	9.364	21.56	9.372	15.95
Propyl gallate	10.167	10.208	0.31	10.176	0.39	10.189	0.66	10.200	0.71
4′,7-Dihydroxyiso-flavone	10.421	10.346	0.62	10.413	1.55	10.412	0.03	ND	–
Quercetin	10.608	ND	–	10.589	2.04	10.623	4.65	10.579	2.17
Cinnamic acid	11.095	11.144	0.029	11.092	0.22	11.109	0.02	11.116	0.02

*Not detected..

Table 3 Comparative HPLC study of major detected compounds in *Moringa oleifera* cultivated in Egypt and other countries.

		Extracts and/or fractions of *Moringa oleifera*					
		Egypt			Indonesia	Tunisia	Saudi Arabia
Moringa oleifera L. leaves extract or fraction	Compound	Methylene Chloride fraction	Ethyl acetate fraction	Butanol fraction	Ethyl acetate [11]	Aqueous Methanolic extract [12]	Methanolic extract [13]
Major compounds detected	Naringenin	Minor	21.56	15.95	–	0.0016	–
	Syringic acid	6.84	9.28	Minor	–	–	–
	Quercetin	2.04	4.65	2.17	0.0017	0.011	0.037
Concentration (mg/g)	Gallic acid	1.94	5.02	15.42	–	0.011	0.048
	Ellagic acid	Minor	120.15	–	–	–	–
	Chlorogenic acid	Minor	Minor	12.75	–	–	–
	Rutin	–	Minor	10.34	–	6.18	0.55

Table 4 Comparative study of GC/MS analysis of FAMEs of seed oil obtained by three different methods of extraction

Compound identified	GC/MS analysis of FAMEs of seed oil obtained by cold compression		GC/MS analysis of FAMEs of seed oil obtained by traditional hexane extraction		GC/MS analysis of FAMES of seed oil obtained by ultrasonic assisted extraction	
	Area %	Retention time	Area %	Retention time	Area %	Retention time
Tetradecanoic acid methyl ester (myristic acid)	0.65	11.26	–	–	–	–
Pentadecanoic acid,14-methyl, methyl ester	–	–	0.36	11.22	–	–
Methyl hexadec-9-enoate	0.62	15.69	–	–	–	–
9-Hexadecenoic acid methyl ester, (z) (palmitoleic acid; omega 7 fatty acid)	0.62	15.56	4.11	15.59	3.38	15.79
Hexadecanoic acid methyl ester (palmitic acid)	15.73	16.81	–	–	14.77	16.44
Hexadecanoic acid-14-methyl, methyl ester	–	–	0.3	32.86	–	–
Heptadecanoic acid methyl ester (margaric acid)	–	–	–	–	0.17	32.87
Methyl-9-cis,11-trans- octadecadienoate	14.42	20.59	–	–	–	–
Octadecanoic acid methyl ester (stearic acid)	13.9	22.28	–	–	–	–
6-Octadecenoic acid methyl ester, (z) (petroselinic acid)	–	–	9.71	20.55	–	–
13-Octadecenoic acid methyl ester, (z)	–	–	51.37	20.89	0.3	20.20
Trans-13-Octadecenoic acid methyl ester	–	–	–	–	5.27	20.55
9-Octadecenoic acid methyl ester, (z) (oleic acid, omega-9 fatty acid)	–	–	–	–	64.78	21.17
Eicosanoic acid methyl ester (arachidic acid)	2.01	25.42	3.28	25.20	–	–
Cis-13-Eicosenoic acid methyl ester (Paullinic acid; omega 7 fatty acid)	–	–	2.03	24.63	–	–
Methyl-9-Eicosenoate (gadoleic acid, omega 11 fatty acid)	–	–	0.57	24.75	–	–
Docosanoic acid methyl ester (behenic acid)	1.81	29.28	3.10	29.21	1.82	29.26
Cis-11-Eicosenoic acid methyl ester (gondoic acid; omega-9 fatty acid)	–	–	–	–	1.28	24.70
% of unsaturated fatty acids	15.66		67.79		75.01	

FAME, fatty acid methyl ester; GC/MS, gas chromatography–mass spectrometry.

Biological investigation

Evaluation of hepatoprotective activity

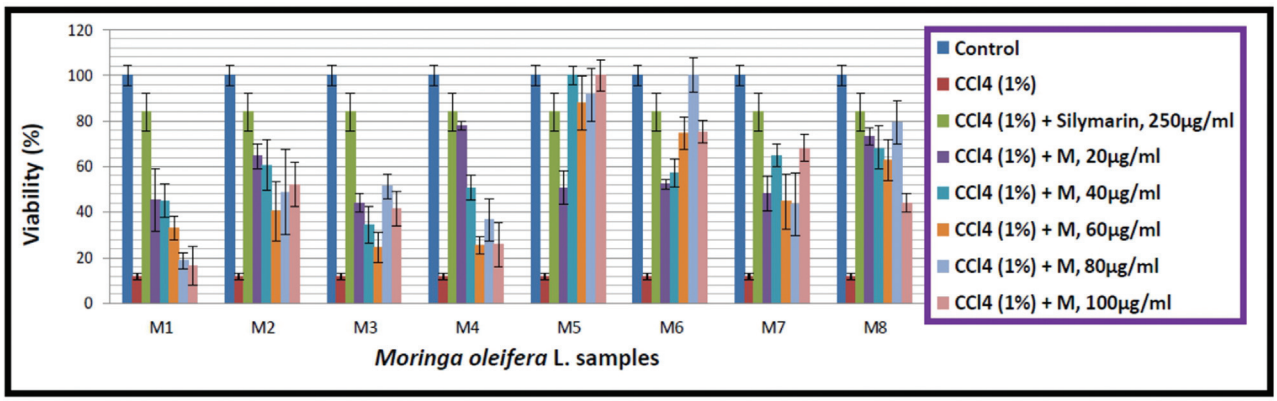
M1–M8 extracts and/or fractions were evaluated for their hepatoprotective activity, and the obtained results revealed the following: petroleum ether and methylene chloride fractions (M5 and M6, respectively) exhibited hepatoprotective activity at concentrations ranging from 40 to 100 µg/ml for M5 and at concentration 80 µg/ml for M6 fraction. The obtained results are summarized in Table 5 and Fig. 1. The results revealed low toxicity and high hepatoprotection activity for petroleum ether and methylene chloride fractions (M5 and M6, respectively). This means that the IC50 of these fractions was more than 100 µg/ml as the concentration that causes 50% cell death was more than 100 µg/ml, so it gives high safety margin for both plant fractions. Both fractions at different concentrations showed hepatoprotective activity exceeding the positive reference for hepatoprotection (silymarin).

Table 5 Hepatoprotective effect of different concentrations of *Moringa oleifera* L. extracts and fractions

	SD%	M1	M2	M3	M4	M5	M6	M7	M8	CCl_4 (1%) +Silymarin, 250 µg/ml
Control	Control	100	100	100	100	100	100	100	100	100
CCl_4 (1%)	CCl_4 (1%)	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7	11.7
CCl_4 (1%)+ M, 20 µg/ml	CCl_4 (1%)+	45.1	64.5	44	77.9	50.8	52.3	48.2	73.3	
CCl_4 (1%)+ M, 40 µg/ml	CCl_4 (1%)+	44.9	60.6	34.5	50.6	100	57	64.9	68.3	
CCl_4 (1%)+ M, 60 µg/ml	CCl_4 (1%)+	33.1	40.4	24.	25.3	87.6	74.5	44.6	62.8	83.9
CCl_4 (1%)+ M, 80 µg/ml	CCl_4 (1%)+	18.5	48.8	51.2	36.6	91.4	100	43.5	79.4	
CCl_4 (1%)+ M, 100 µg/ml	CCl_4 (1%)+	16.5	52.	41.6	25.8	100	75.2	68.3	44	

*Not detected.

Figure 1



Viability of monolayer of HepG2 cells after treatment with different concentrations of *Moringa oleifera* L. samples and 1% CCl_4 in comparison with silymarin as control using MTT colorimetric assay.

These results come in accordance with the results obtained from a previous study [26], which also stated that n-hexane and dichloromethane fractions (of *M. oleifera* L. leaves, also cultivated in Egypt) have the most potent hepatoprotective effect among other fractions and extracts. So, future pharmacological and pharmaceutical studies should be conducted to make available drug formulations for liver protection.

Evaluation of cytotoxic activity

M1–M8 extracts and/or fractions were evaluated for their cytotoxic activity, and the obtained results revealed the following:

Petroleum ether fraction, total 80% methanolic extract, and methylene chloride fraction (M5, M3, and M6, respectively) exhibited very high cytotoxic activity represented by IC50 values (19.25, 21.09, and 21.52 µg/ml for M5, M3, and M6, respectively). The obtained results are summarized in Table 6 and Fig. 2.

Previously reported studies indicated *M. oleifera* L. leaf aqueous extract exhibits cytotoxic activity on BHK-21 and HepG2 cell lines, respectively [27,28]. In our study, treatment with 80 µg/ml of *M. oleifera* leaf aqueous extract (M4) produced inhibition ratios get close to 80%, whereas in the previous reported study [28], this effect was produced by 200 µg/ml of *M. oleifera* leaf extract. Further studies are required to make optimum use of these valuable plant fractions as cytotoxic agents, especially the plant cultivated in Egypt.

Table 6 Cytotoxic effect of different concentrations of *Moringa oleifera* L. extracts and fractions on HepG2 cells

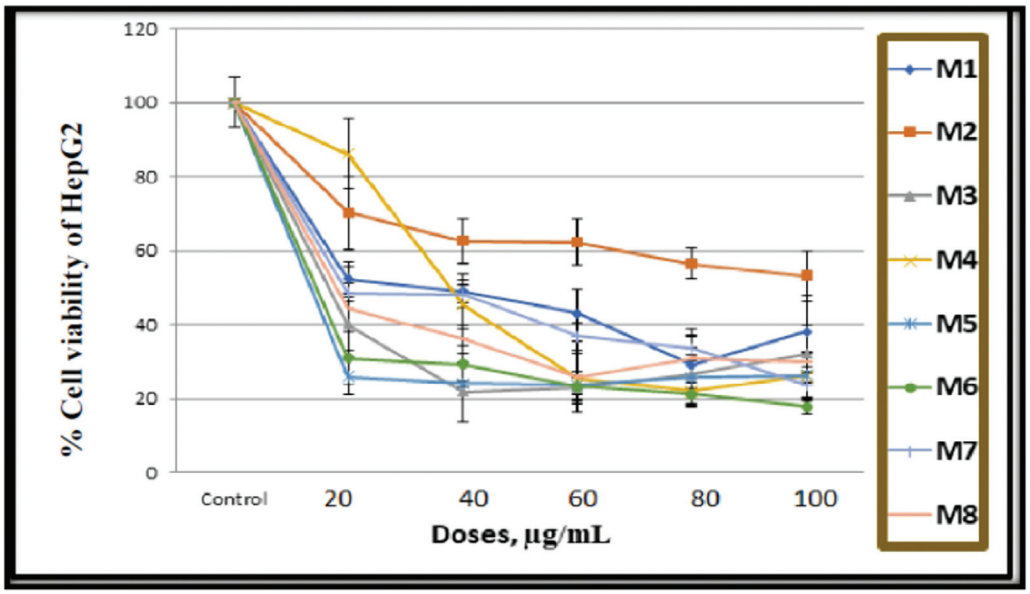
	M1	M2	M3	M4	M5	M6	M7	M8
Control	100	100	100	100	100	100	100	100
20 µg/ml	52.3	70.3	39.9	86.2	25.9	30.9	48.7	44.3
40 µg/ml	49.1	62.6	21.8	45.3	24.1	29.2	48.3	36
60 µg/ml	43.1	62.3	22.7	25.3	23.7	23.1	36.9	26
80 µg/ml	29.1	56.4	26.6	22.1	25.8	21.3	33.5	30.8
100 µg/ml	38.1	53.1	32	26.2	26.3	17.8	23.6	29.7
IC50	35.1	>100	21.1	37.9	19.2	21.5	34.7	25.7

Antibacterial effect

The fresh leaves' water extract and the 100% ethanol extract were tested for their antibacterial activity, and results are compiled in Table 7.

The tested *M. oleifera* L. extracts showed antibacterial activity against *Tannerella forsythia* (a gram-negative bacteria) that exceeded positive control. Moreover, the

Figure 2



Viability of monolayer of HepG2 cells after treatment with different concentrations of *Moringa oleifera* L. samples using MTT colorimetric assay.

Table 7 The antibacterial activity of different *Moringa oleifera* L. extracts

Bacteria	Zone of inhibition (mm)		
	Aqueous extract	100% ethanol extract	Positive control (Imipenem)
Gram-negative bacteria			
Escherichia coli	11	23	27
Coliform spp.	9	15	17
Klebsiella spp.	7	16	20
Tannerella forsythia	19	19	17
Prevotella intermedia	10	14	21
Pseudomonas spp.	14	16	19
Aggregatibacter actinomycetemcomitans	14	20	21
Gram-positive bacteria			
Staphylococcus aureus	16	14	16
Bacillus cereus	10	11	14
Streptococcus-B haemolytica	12	17	31

Values are presented as mean. Diameter of inhibition zone including diameter of disc 6 mm. Minimum inhibitory concentration (values in µl disc-1).

80% ethanolic extract showed antibacterial activity against *Bacillus cereus* (a gram-positive bacteria), which also exceeded the positive control (Imipenem). In addition, the 80% ethanolic extract of leaf of *M. oleifera* L. showed the maximum diameter of zone of inhibition against *Staphylococcus aureus*, that is, 26 mm, indicating a potent source of new antibiotic alternative.

This study showed that the extract of *M. oleifera L.* is active against the tested gram-positive and gram-negative microorganisms, and the results confirm the use of the plant in traditional medicine. Ethanol extract exhibited a higher degree of antimicrobial activity as compared with aqueous extract. However, further work is needed to isolate the secondary metabolites from the extract to test for specific antimicrobial activity. This in-vitro study demonstrated that folk medicine could be as effective as modem medicine to combat pathogenic microorganisms. *M. oleifera* L. represents an economical and safe alternative to treat infectious diseases, and it has many other uses. Its use is thus highly encouraged, and more work should be carried out to determine the antibacterial constituents that can be used for drug formulation.

Conclusion

M. oleifera seed oil is highly recommended owing to its proven health properties, which was emphasized by this FAMES GC/MS analysis, which revealed the presence of oleic acid in an imposing amount,

especially in oil obtained by ultrasonic extraction technique. The biological evaluation (hepatoprotective, anticancer, and antibacterial activities) for the plant different extracts and fractions revealed positive test results for certain extracts and fractions, so further studies should be done to make optimum use of these medicinal plant as drug alternative. In conclusion, the oil of *M. oleifera* is interesting product for its nutritional composition and its biological activities. Its use could have a positive effect on the nutritional status and health of people of developing countries.

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Conflicts of interest
There are no conflicts of interest.

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