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Chemical profile, antimicrobial and antioxidant activities of *Moringa oleifera* Lam leaves grown in Saudi Arabia

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Abstract

Background: Moringa tree (family Moringaceae) commonly known as the miracle tree. *Moringa oleifera* Lam is an important species belongs to the genus Moringa.

Objective: To investigate the chemical composition, antimicrobial and antioxidant activities of *Moringa oleifera* leaves grown in KSA.

Methods: Gas chromatography–mass spectrometry (GC/MS) analysis was carried out for identification of the chemical composition of the extract. Disk agar and phosphomolybdenum methods were used to evaluate the antimicrobialand antioxidant activities.

Results: GC/MS analysis revealed a total of thirty-seven phytochemical constituents, with the major constituents found to be 9,12,15-Octadecatrienoic acid, methyl ester (47.89%), Pentadecanoic acid, methyl ester (22.01%), 9,12-Octadecadienoic acid, methyl ester (10.37%), Octadecanoic acid, methyl ester (3.47%), 10-Methyl-E-11-tridecen-1-ol-propionate (3.01%), Hexatriacontane (1.61%), Tetracosanoic acid, methyl ester (1.21%), and Eicosanoic acid, methyl ester (1.06%).The antimicrobial assay, using standard microbiological methods, showed that methanol; dichloromethane, ethyl acetate and n-butanol extracts have considerably higher antimicrobial activity with inhibition zones ranged from 7 to 12mm, and there is no any activity was recorded with petroleum ether extract. In phosphomolybdenum assay, the *n*-BuOH extract exhibited the highest total antioxidant capacity (TAC) value with 336.13 mg AAE /g dry extract, followed by 327.71, 220.03, 137.63 and 71.15for ethyl acetate, methanol, dichloromethane and petroleum ether extracts, respectively.

Conclusion: Moringa oleifera leaves may be a good source of naturally occurring antimicrobial and antioxidants agents.

Keywords: *Moringa oleifera*; Chemical profile; GC/MS; Antimicrobial activity; Antioxidant activity.

1. INTRODUCTION

Free radicals or reactive species are highly energetic molecules with odd electron able to reacts with cells and tissues. Accumulation of such species in our bodies led to generation of oxidative stress, which is associated with several health problems like cancer, inflammations, and cardiovascular diseases [1,2]. Several reports have revealed that many plants produce secondary metabolites with antioxidant and shown powerful free radical scavenging potentials [3-5].The antioxidant activity of crude extracts returns to the presence of phenolic groups having unique mode of action and a characteristic structural criteria for effective free radical scavenging activity [6,7].

Microbial infections still a great public issue and there is a dramatic increasing in the microbial resistance to the existence antimicrobial agents [8]. So, scientists do their best efforts to develop new anti-microbial agents from the natural resources to treat the infectious disease. Traditionally, a wide spectrum of plant secondary metabolites were isolated and identified from edible plants that can be used to treat abundant of infectious disease with reduced side effects [4].

Moringa is widely distributed in different climates including tropical and subtropical regions [9]. *M. oleifera* have a wide range of medicinal uses and nutritional values [10]. Previous reports revealed that the plant contains minerals, protein, vitamins, amino acids, polysaccharide and *β*-carotene [11-14]. Traditionally different parts of *M. oleifera* have been used in the treatment of severalailments and health problems [15]. It was reported that the plant possess a wide range of biological activities like; antitumor [16], antimicrobial [17], hepatoprotective [18] and antioxidant [19]. A literature survey indicatedthat the presence off lavonol glycosides [20] and terpenes [21]. Hamed et al., [9] reported the isolation of coumaric acid 4-*O*-(2'-*O*-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside, chlorogenic acid, niazirin, 3,4-dihydroxy-*β*-phenylethoxy-*O*-α-L-rhamnopyranosyl-(l→2)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-*β*-D-glucopyranoside, gallic acid, taxifolin and benzyl-carbamo-thioethionate [9]. Therefore, the aims of the current study were to determine the chemical composition of the methanolic extract of *Moringa oleifera* leaves and to evaluate the antimicrobial and antioxidant activity of different solvent extracts.

2. EXPERIMENTAL AND METHODS

2.1. Plant materials

Plant leaves were collected from Al Muzahimiyah, Kingdom of Saudi Arabia during February 2018. The plant was identified by Dr. Mona S. Alwahibi, Associate Professor of Molecular Plant Taxonomy, King Saud University, KSA.

2.2. Extraction and fractionation

The dried leaf was grinded and extracted with methanol at room temperature for four days (8×500 ml). The extracts were evaporated under vacuum until dryness. The obtained residue was further undergoes fractionation using petroleum ether, methylene chloride, ethyl acetate and *n*-butanol.

2.3. Determination of total antioxidant capacity

The antioxidant activity of the plant extract was determined according to phosphomolybdenum method, using ascorbic acid as standard. In this method, 0.5 ml of each extract (200 µg /ml) in methanol was combined in dried vials with 5 ml of reagent solution (0.6 M sulfuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate). The vials containing the reaction mixture were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled at room temperature, the absorbance was measured at 695 nm against a blank. The blank consisted of all reagents and solvents without the sample and it was incubated under the same conditions. All experiments were carried out in triplicate. The antioxidant activity of the extracts was expressed as the number of equivalents of ascorbic acid (AAE) [22,23].

2.4. Evaluation of antimicrobial activity

2.4.1. Disc agar diffusion method

Disc agar plate method was used to estimate the antimicrobial activities treated fabrics [24]. Six different test microbes; *Staphylococcus aureus* (G+ve bacteria), *Pseudomonas aeruginosa* (G-ve bacterium), *Candida albicans* (yeast) and the fungi (*Aspergillus niger*) were selected to evaluate the antimicrobial activities. The bacterial and yeast test microbes were grown on a nutrient agar medium (DSMZ1) of the following components (g/l): Peptone (5.0), Meat extract (3.0), Agar (20.0), distilled water (1000.0 ml) and the pH to 7.0. On the other hand, the fungal test microbe was cultivated on Potato-Dextrose agar (PDA) medium (DSMZ129) of the following ingredients (g/l): Infusion from 200g potatoes, glucose (20), distilled water (1000.0ml) and the pH was recorded to be 6. The culture of each test microbe was diluted by distilled water (sterilized) to about 10^6 - 10^8 cells/ml, then 100 microliter of each was spreaded over the appropriate solidified plates using sterilized swab. Filter paper discs each was saturated with 200microgram sample (5 mm diameter) were placed on the surface agar plates seeded with test microbes and incubated for 24 hrs at the appropriate temperature of each test organism. Antimicrobial activities were recorded as the diameter of the clear zones (including the film itself) that appeared around the films.

2.4.2. GC/MS Analysis

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadrupole MS and TGSMS Fused Silica Capillary Column (30m, 0.251 mm, 0.1 mm Film thickness), National Research Center, Giza, Egypt. For GC/MS detection, an electron ionization system with ionization energy for 70 ev was used as the carrier gas at a constant flow rate of 1ml/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature 40°C (hold 3 min) to 280°C was a final temperature at an increasing rate of 5°C /min (hold 5 min). The identified components were investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY Library data of the GC/MS system [25,26].

3. RESULTS AND DISCUSSION

3.1. Identification of chemical constituents by GC/MS analysis

GC/MS analysis of the total extract of *M. oleifera* leaves revealed the presence thirty-seven compounds **(Figure 1 and Table 1)**, representing (98.64%) of the total composition. 9,12,15-Octadecatrienoic acid, methyl ester (47.89%), Pentadecanoic acid, methyl ester (22.01%), 9,12-Octadecadienoic acid, methyl ester(10.37%), Octadecanoic acid, methyl ester (3.47%), 10-Methyl-E-11-tridecen-1-ol-propionate (3.01%), Hexatriacontane (1.61%), Tetracosanoic acid, methyl ester (1.21%), and Eicosanoic acid, methyl ester (1.06%)were the major identified components **(Figure 2)**.

GC/MS analysis of *M. oleifera* leaves from two Indian locations (Madurai& Chennai) was performed. The results revealed that 1,30-triacontanediol (14.98%), octacosane (8.57%), Z-14-nonacosane (8.3%) and 2,2-dimethyl-1-oxa- 2-silacyclotrid ecanone-13 (8.28%) were the major compounds in leaves from Madurai. While, nonacosane (15.55%) and gamma-sitosterol (9.56%) were the major compounds in leaves from Chennai [27]. Mahdi et al., [28] reported that the GC/MS profiling of dichloromethane fraction of *M. oleifera* leaf ethanol extract showed the presence of eight compounds identified as: limonene, (1S,6R)-γ-himachalene(4aS,7S)-7-isopropyl-4a-methyloctahydro-2(1H) naphthalenone, hexadecanoic acid, ethyl ester, phytol, 9,12,15-octadecatrienoic methyl ester, 4-(2-furyl)-6-(1-piperidinyl)-1,3,5-triazin-2-amine and α-tocopherol [28].

In another study carried out by Jayanthi et al., [29] GC/MS analysis of the methanolic extract of *M. oleifera* leaves revealed presence of 9,12,15-octadecatrienoic acid ethyl ester, 6-octadecenoic acid, cis-vaccenic acid and 2-octyl-cyclopropaneoctanal [29]. Also, ethyl oleate, quinic acid and cis-9-hexadecenal were detected as major constituents in the methanol extract of *Moringa oleifera* flowers growing in India [30].

Moreover, methyl (11E)-11-octadecanoate and *cis*-octadecanoic acid were recorded as major constituents in the methanolic extract of *M. oleifera* leavesgrowing in Nigeria [31]. GC/MS analysis of petroleum ether extract of *M. oleifera* roots revealed that it contained major compounds namely; trans-13-docosene (37.9%), nonacosane (32.6%), cycloartenol (28.6%), nonadecanoic acid (13.9%) and cyclooctasulfur S8 (13.9%). While, investigation of dichloromethane extract led identification ofmajor constituents namely; nasimizinol (58.8%), oleic acid (46.5%), N-benzyl-N-(7-cyanato heptanamide (38.3%), N-benzyl-N-(1-chlorononyl) amide (30.3%), bis [3-benzyl prop-2-ene]-1-one (19.5%) and N,N-dibenzyl-2-ene pent 1,5 diamide (11.6%) [32].

3.2. Antimicrobial activity

The antimicrobial activity was evaluated via disk agar diffusion method (100 microliter per cup) using four pathogenic bacterial & fungal strains e.g. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*. The results in Tables (2) showed that the tested extracts exhibited antimicrobial activity against the three strains expressed by inhibition zones which ranged from 0 to 10mm (*S. aureus*),from 0 to 12mm (*P. aeruginosa*) and from 0 to 11mm (*C. albicans*) and there is no any activity was recorded against *A. niger*, relative to two standard antibiotics namely; Neomycinwith inhibition zones which ranged from 17 to 21mm and Cyclohexamidewith inhibition zone 25mm.The different extracts of *M. oleifera* leaves showed strong antimicrobial activity, which may be return to the synergistic action (Co-activity) of their chemical constituents especially flavonoids and phenolic acids [33].

Reviewing the literature revealed that, the growth of some pathogenic microbial strains viz., *E. coli*, *S. typhi*, *S. typhimurium*, *S. dysentri*, *S. flexneri* and *S. aureus* was inhibited using*M. oleifera* leaves from two Indian locations (Madurai & Chennai) [34]. The antimicrobial activities of different extracts from*M. oleifera* leaves, flower and seeds wereinvestigated *in vitro* against clinical pathogens like *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp, *Proteusmirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus.* Theresults revealed that the ethanolic extracts demonstrated the highest activity, while the aqueous extracts showed the least activity against *S. typhi* [35]. Furthermore, the ethanol extract of *M. oleifera* grown in Taiwan showed *in vitro* anti-fungal activities against dermatophytes such as *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton Xoccosum*, and *Microsporum canis* [36].

3.3. Antioxidant activity

The total antioxidant capacity (TAC) of the tested extracts was evaluated via phosphomolybdenum antioxidant assay. This assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green colored [phosphate=Mo (V)] complex at acidic pH with a maximal absorption at 695 nm [37,38]. In this assay, the (TAC) value of the most active *n*-BuOH extract was 336.13mg AAE /g dry extract, followed by 327.71, 220.03, 137.63 and 71.15mg AAE /g dry extract for EtOAc, methanol, dichloromethane and petroleum ether extracts, respectively **(Table 3)**.

Polyphenolic compounds such as flavonoids, phenolic acids and tannins are considered to be the major contributors to the antioxidant activity of medicinal plants, fruits and vegetables. The antioxidant activities of polyphenols were attributed to their redox properties, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers, as well as their metal chelating abilities [39].

The total antioxidant activity of *M. oleifera* leaves grown in Chad, Southwestern Algeria and Haiti was evaluated via ABTS assay. The results revealed that the TEAC values are 304.63, 427.16 and 335.61 µmol trolox/g, respectively for leaves from Chad, Southwestern Algeria and Haiti [37]. In another study, the antioxidant activity of the aqueous extract of *M. oleifera* leaves grown in India was determined via DPPH technique. The results showed that the DPPH scavenging activity was 55.1% at 100 mg/ml of the aqueous extract compared to 83.42% for ascorbic acid [34]. Moreover, Hamed et al (2017) reported that the total antioxidant capacity (TAC) values of the different solvent extracts of *M. oleifera* leaves grown in Egypt were 316.43,

203.35, 181.56, 86.70, 76.62 and 50.83 mg ascorbic acid equivalent/g dry extract; respectively for butanol, ethyl acetate, 85% methanol, water, dichloromethane, and petroleum ether extracts [9].

4. CONCLUSION

In this work, 37compoundswere identified in the methanol extract of *M. oleifera* leavesgrown in KAS using GC/MS technique. The identified compounds contained mono, sesqui, di and tri- terpenes and their oxygenated derivatives. Moreover, the tested extracts showed noticeable antioxidant and antimicrobial activities which may be due to the presence of the identified compounds. In conclusion, *M. oleifera* leaves are considered a promising source of natural antimicrobial and antioxidant agents.

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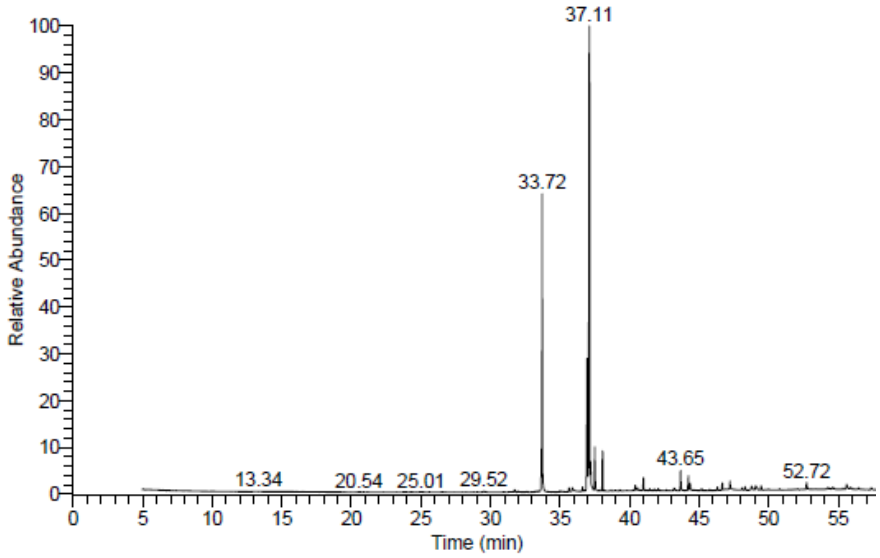
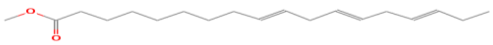
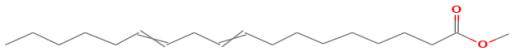


Fig. 1. Gas chromatography-mass spectrometry (GC/MS) chromatogram of the total extract of *M. oleifera* leaves

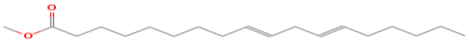
i. Pentadecanoic acid, methyl ester



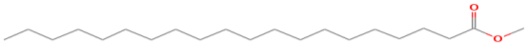
ii. 9,12,15-Octadecatrienoic acid, methyl ester



iii. 9,12-Octadecadienoic acid, methyl ester

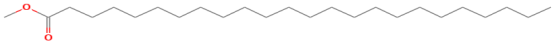


iv. Octadecanoic acid, methyl ester



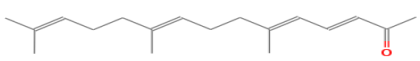
v. Eicosanoic acid, methyl ester

vi. Hexatriacontane

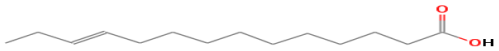


vii. Tetracosanoic acid, methyl ester

viii. Eicosane



ix. (E,E)-farnesylaceton



i. E-11-Tetradecenoic acid

ii. Pentacosane

Fig. 2. Chemical structures of some identified compounds in the total extract of *M. oleifera* leaves

Table 1.Chemical composition of the total extract of *M. oleifera* leaves

No	R_t.	Area %	M.W.	M.F.	Identified compounds[1]
1	13.0	0.07	131	$C_6H_{13}N_S$	N-Methyl-à-diMethylThioPropylamide
2	29.52	0.16	242	$C_{15}H_{30}O_2$	Tetradecanoic acid, methyl ester
3	31.73	0.23	296	$C_{20}H_{40}O$	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
4	31.95	0.13	178	$C_{12}H_{18}O$	6-Dodecanone
5	33.27	0.12	268	$C_{17}H_{32}O_2$	7-Hexadecenoic acid, methyl ester
6	33.45	0.08	222	$C_{15}H_{26}O$	Farnesol
7	33.71	22.01	256	$C_{16}H_{32}O_2$	Pentadecanoic acid, methyl ester
8	35.01	0.16	242	$C_{15}H_{30}O_2$	Pentadecanoic acid
9	35.63	0.40	284	$C_{18}H_{36}O_2$	Hexadecanoic acid, 15-methyl, methyl ester
10	36.62	0.35	334	$C_{22}H_{38}O_2$	Cyclopropaneoctanoic acid, 2-[[-2-[(-2-ethylcyclopropyl)-methyl]-cyclopropyl]-methyl], methyl ester
11	36.95	10.37	294	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester
12	37.11	47.89	292	$C_{19}H_{32}O_2$	9,12,15-Octadecatrienoic acid, methyl ester
13	37.20	0.81	296	$C_{20}H_{40}O$	Phytol
14	37.50	3.47	298	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester
15	38.06	3.01	268	$C_{17}H_{32}O_2$	10-Methyl-E-11-tridecen-1-ol-propionate
16	38.68	0.11	310	$C_{22}H_{46}$	Docosane
17	39.28	0.09	256	$C_{16}H_{32}O_2$	Tetradecanoic acid, 12-methyl, methyl ester
18	40.41	0.42	422	$C_{30}H_{62}$	Triacontane
19	40.56	0.18	254	$C_{16}H_{30}O_2$	9-Hexadecenoic acid
20	40.99	1.06	326	$C_{21}H_{42}O_2$	Eicosanoic acid, methyl ester
21	41.45	0.18	292	$C_{21}H_{40}$	Cyclohexane,1,1'-[1-(2,2-dimethylbutyl)-1,3 propanediyl]bis
22	41.78	0.16	262	$C_{18}H_{30}O$	(E,E)-farnesylacetone
23	42.06	0.17	352	$C_{25}H_{52}$	Pentacosane
24	43.19	0.20	324	$C_{23}H_{48}$	Heptadecane, 9-hexyl
25	43.66	1.61	506	$C_{36}H_{74}$	Hexatriacontane
26	44.22	1.21	382	$C_{25}H_{50}O_2$	Tetracosanoic acid, methyl ester
27	44.35	0.68	390	$C_{24}H_{38}O_4$	1,2-Benzenedicarboxylic acid, dioctyl ester
28	45.18	0.20	282	$C_{20}H_{42}$	Eicosane
29	45.74	0.15	270	$C_{17}H_{34}O_2$	Pentadecanoic acid, 14-methyl, methyl ester
30	46.31	0.29	152	$C_{10}H_{16}O$	Limonene oxide
31	46.66	0.55	268	$C_{19}H_{40}$	Heptadecane, 2,6-dimethyl-
32	48.08	0.19	214	$C_{14}H_{30}O$	1-Tetradecanol
33	48.29	0.31	290	$C_{20}H_{34}O$	trans-Geranylgeraniol
34	48.77	0.51	240	$C_{17}H_{36}$	Tetradecane, 2,6,10-trimethyl
35	49.06	0.66	226	$C_{14}H_{26}O_2$	E-11-Tetradecenoic acid
36	49.46	0.34	450	$C_{32}H_{66}$	Docosane, 11-decyl
37	50.0	0.11	296	$C_{19}H_{36}O_2$	Cyclopentanetridecanoic acid, methyl ester
T%		98.64%			

[1]Compounds identified via comparison its mass spectrum with NIST library, Adams, 2001 and literature. M.F.: Molecular formula; M.W.: Molecular weight; R_t.: Retention time.

Table 2. Antimicrobial activity (inhibition zones) of the different solvent extracts of *M. oleifera* leaves

Sample	Clear zone (ϕmm)			
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
MeOH	10	12	11	0
Pet. ether	0	0	0	0
CH_2Cl_2	7	8	9	0
EtOAc	9	11	8	0
n-BuOH	8	10	8	0
Neomycin[1]	17	20	21	0
Cyclohexamide[2]	0	0	0	25

[1]Neomycin was used at 200 microgram per disk.
[2]Cyclohexamidewas used at 200 microgram per disk.

Table 3. Total antioxidant capacity (TAC) of different solvent extracts of *M. oleifera* leaves

Sample	Total antioxidant capacity (mg AAE /g dry ext.)[1]
MeOH	220.03 ± 3.06
Pet. ether	71.15± 1.62
CH_2Cl_2	137.63 ± 2.80
EtOAc	327.71 ± 4.29
n-BuOH	336.13 ± 1.65

[1]*Total antioxidant capacity (TAC) monitored by the phosphomolybdenum assay expressed as mg ascorbic acid equivalent AAE /g dry extract.*