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GC-MS analysis of *Mirabilis japala* L. root extract and studying its antimicrobial and antioxidant activity

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Abstract

Mirabilis jalapa L., a tropical plant widely grown as a medicinal plant worldwide, was studied for the antimicrobial and antioxidant activities of its root extract. Methanolic (MMRE) and aqueous (AMRE) extracts of *Mirabilis jalapa* L. root were prepared and tested at concentrations of 2000, 4000, and 6000 ppm for antimicrobial activity against *Salmonella* spp., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* (E. coli), *Leuconostoc* spp., *Bacillus thuringiensis*, *Aspergillus niger*, *Penicillium* spp., and *Rhizopus oryzae*. Both MMRE and AMRE exhibited inhibitory activity against the tested organisms, with inhibition zone diameters increasing with higher extract concentrations. The highest inhibition zones at 6000 ppm of MMRE were 19.3 mm and 41.7 mm against *Bacillus subtilis* and *Aspergillus niger*, respectively. GC–MS analysis was used to identify the bioactive compounds in MMRE and AMRE. Antioxidant capacity was evaluated based on the DPPH free radical scavenging activity of the extracts. Among the tested concentrations, 250 mg/ml of MMRE and AMRE showed the highest scavenging activities of 96% and 89%, respectively.

1. Introduction

Historically, medicinal plants have played a significant role worldwide in preventing and controlling diseases. Human health has undoubtedly improved on a global scale as a result of the extensive usage of medicinal plants [1]. Previous studies have demonstrated the value of plant extracts in preventing or suppressing a variety of illnesses in addition the antioxidant activity of plant extracts for human health and their ability to resist microbes and other infections [2-8].

Mirabilis jalapa L., classified within the family *Nyctaginaceae*, is well-known as the garden “four o’clock” and is traditionally used to treat ailments such as diarrhea, gastrointestinal disorders, and infectious ulcers [9]. Chemical analyses of several *M. jalapa* components have confirmed the presence of biologically active compounds [10, 11]. In vitro studies reported that extracts from various plant parts exhibited antifungal activity against *Daedaleaflavida*, *Candida albicans*, and *Aspergillus nigricans* [12–15]. Carbohydrates, flavonoids, alkaloids, steroids, glycosides, terpenes, unstable oils, alcohols, fatty acids, and a wide range of trace elements were reported to be present in *Mirabilis jalapa* L. [16]. Earlier medical reports indicate that *M. jalapa* L. has been applied to treat abdominal, cardiac, cytotoxic, antibacterial, hypolipidemic, anti-inflammatory, nervous system, and many other medical conditions [17].

In addition, the substantial scavenging potential of methanolic extracts from various parts of *M. jalapa* plant has been identified. Furthermore, lab animal studies (namely rats) have shown that ethanol extract of *M. jalapa* roots enhances spermatogenesis and improves fertility [13]. According to [18, 19], *M. jalapa* can reduce

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glucose, blood lipids, and blood pressure, thereby supporting heart health. GC–MS (Gas Chromatography–Mass Spectrometry) has been employed to estimate biologically active substances in medicinal plants due to its utility in profiling bioactive volatile compounds and essential oils [20, 21].

The aim of this study was to investigate the active components of *M. jalapa* root extracts and evaluate their antibacterial and antioxidant activities against specific microorganisms, given the limited studies on its active compounds and its well-known therapeutic value.

2. Material and Methods

2.1. Sample collection

Mirabilis jalapa plant roots were collected from Karbala province, washed, dried overnight at 50°C, powdered, and stored in a refrigerator for further use.

2.2. Extraction of *Mirabilis jalapa* L. roots

The methanolic extract of *M. jalapa* plant roots (MMRE) was prepared according to the method described by [19] with some modifications. Specifically, 50 g of dried, powdered *M. jalapa* roots were mixed with 290 ml of 80% methanol, and the mixture was then filtered using a vacuum pump. Three to four additional methanol extractions were performed on the residue until complete extraction was achieved. The methanol was evaporated to dryness using a rotary vacuum evaporator at 50°C. The methanol-free extract was lyophilized and stored in a dark place until use.

The aqueous extract of *M. jalapa* roots (AMRE) was prepared by placing 50 g of root powder in 200 ml of sterilized water and a Soxhlet apparatus was applied for the extraction for 24 hours. The mixture was then filtered through Whatman No.1 filter paper and then was concentrated. The pure extract was stored at 4°C until further use.

2.3. Evaluation of MMRE and AMRE antimicrobial inhibition activity

Several types of microorganisms (*Salmonella* spp., *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Leuconostoc* spp., *Bacillus thuringiensis*, *Aspergillus niger*, *Aspergillus* spp., *Penicillium* spp., and *Rhizopus oryzae*) were obtained from the Department of Food Science, College of Agricultural Engineering, University of Baghdad, Baghdad, Iraq. The inoculum suspension of the pathogens was spread on Mueller–Hinton agar and PDA agar plates and allowed to dry for approximately 15 minutes [22]. For each isolate, a sterile steel borer was used to create a 25 µl well, which was then filled with MMRE and AMRE. The plates were incubated at 37°C for 24 h for bacteria and at 25°C for 5 days for fungi. After incubation, the inhibition zones (mm) were measured according to the method described by [23].

2.4. Investigation of *Mirabilis jalapa* L. active compounds by GC-MS technique

The GC–MS technique was used to characterize *Mirabilis jalapa* L. under the following conditions: a GC capillary column with dimensions of 50 m × 0.2 mm × 0.3 µm (Carbowax 40M) was employed, helium gas was used as the carrier gas at a flow rate of 30 cm/s, a manual injector was used to inject 1.5 µL of diluted samples

in ethyl alcohol (1:5 v/v) with a split ratio of 200:1 at 150°C [2, 11]. The oven temperature was initially set at 65°C for 20 minutes and then increased gradually at a rate of 2.5°C/min to reach 250°C throughout 50 minutes. Mass spectra were used to identify the compounds by comparing the sample spectra with those stored in the ChemStation public library, which contains approximately 46,000 reference compounds [24].

2.5. Testing the antioxidant activity of MMRE and AMRE

The DPPH radical-scavenging activity of MMRE and AMRE was determined using the method described by [25] with some modifications. A volume of 500 ml of each extract (50–250 mg/ml) was added to 315 ml of 90% alcohol and 175 ml of a DPPH scavenging solution (0.1 mM in methanol). The mixtures were incubated for 20 minutes in the dark at room temperature. The scavenging activity was then measured spectrophotometrically by observing the decrease in absorbance at 517 nm, which diminishes due to the formation of an antiradical complex.

Radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = [(A_{(control)} - A_{(sample)}) / A_{(control)}] \times 100$$

Where:

$A_{(control)}$ is the absorbance of the control

$A_{(sample)}$ is the absorbance of MMRE or AMRE.

2.6. Statistical analysis

The values were reported as mean ± standard deviation and analyzed for variation using SAS software. Significant differences between the control and replicates were determined using Duncan's multiple range test at $P \leq 0.05$ [26].

3. Results and Discussion

A total of 22.5 g of *Mirabilis jalapa* L. root extract was obtained from each 100 g of plant dry matter. The yield of root extracts varied according to numerous factors, including extraction methods, genetic and environmental conditions, and the harvesting period [27].

3.1. Inhibition capability of MMRE and AMRE

Results presented in table (1) showed the inhibition capability of MMRE and AMRE against microbial growth. The highest reported effect against *Bacillus subtilis* occurred at 6000 ppm of MMRE, where an inhibition zone with a diameter of 19.3 mm was recorded compared to other treatments. A direct correlation was recorded between increased concentration and increased effectiveness in both MMRE and AMRE.The antibacterial effect was assessed using the agar diffusion method. Generally, the biological activity of plant extracts is associated with their chemical components, which depend on plant genotype and are affected by a number of factors, including environmental and cultivation conditions [28]. Glycosides, tannins, proteins, various phenolic substances, and alkaloids contained in the roots of *Mirabilis* play a significant role in their antibacterial activity by damaging biological cell membranes [29, 30]. Moreover, plant leaf and root extracts were found to have antibacterial effects against *E. coli* and *Staphylococcus aureus* [30]. Active compounds

present in *Mirabilis* leaves also play an important role in antibacterial activity by causing damage to the cell membrane [31].

Table 1. Inhibition zone (mm) of bacteria by *Mirabilis jalapa*roots methanol and aqueous extracts.

Microorganisms	MMRE			AMRE		
	Inhibition Zone (mm)			Inhibition Zone (mm)		
	2000 ppm	4000 ppm	6000 ppm	2000 ppm	4000 ppm	6000 ppm
Salmonella spp.	7.2	11.2	14.9	6.8	11.2	15
Bacillus subtilis	9.9	15.3	19.3	9.1	14	18.9
Pseudomonas aeruginosa	8.7	12.8	18.1	8.1	11.9	17.0
Escherichia coli	0	10.3	13.4	9	9.1	12.3
Bacillus thuringiensis	8.8	12.5	17.9	7.3	11.4	16.7
Leuconostoc spp.	7.5	9.3	6	0	8.3	14.8

Results in Table 2 showed that the alcoholic extract preceded the aqueous extract in susceptibility inhibition of molds and was more influential against *Aspergillus niger*. The growth diameters were 11.4, 25.3, and 41.7 mm for the alcoholic extract and 10.1, 23.7, and 39.8 mm for the aqueous extract at concentrations of 2000, 4000, and 6000 ppm, respectively. The results showed the same effect of the alcoholic extract against *Penicillium* spp. at concentrations of 4000 and 6000 ppm, with inhibition zones of 20.0 and 37.4 mm, compared with the aqueous extract at the same concentrations, which showed inhibition zones of 16.8 and 29.1 mm, respectively. The zone diameters of the alcoholic extract against *Rhizopus oryzae* at concentrations of 4000 and 6000 ppm were 24.3 and 40 mm, respectively, compared with the aqueous extract, which showed 18.3 and 31.8 mm, respectively. The activity of the extract can be attributed to the occurrence of a high content of flavonoids and sitosterol identified by GC–MS. The inhibition ability of *Mirabilis* root extracts was due to the presence of phenolic mixture types in addition to their subtle acidic role [32].

Table 2. Effect of aqueous and alcoholic extract of *Mirabilis jalapa* roots extracts against some types of fungi.

Microorganisms	MMRE			AMRE		
	Inhibition Zone (mm)			Inhibition Zone (mm)		
	2000 ppm	4000 ppm	6000 ppm	2000 ppm	4000 ppm	6000 ppm
Aspergillus niger	11.4	25.3	41.7	10.1	16.8	39.8
Penicillium spp.	14.8	16.8	37.4	0	24.3	29.1
Rhizopus oryzae	16	23.7	40	8.2	18.1	31.3

3.2. GC-Mass analysis of AMRE and MMRE

The compounds present in AMRE are shown in Table 3 and Figure 1. Two compounds were identified using GC–MS: hexadecanoic acid, methyl ester, and 9-octadecenoic acid, methyl ester. These results explain the antifungal activity of *Mirabilis jalapa* L. The breakdown of mycotoxins could be due to the antibacterial and antifungal activity of the methyl ester fatty acids [32]. Various compounds were detected by GC–MS analysis of MMRE, as shown in Table 4 and Figure 2. The biological and anti-stress activities of *Mirabilis*

jalapa L. extracts could be due to the presence of phytochemical compounds such as alkaloids, saponins, flavonoids, tannins, and phenols [16].

Table 3. Constitution of *Mirabilis* aqueous extract samples by GC-MS.

Peak No.	Compound	Retention time (RT)	Area %	Similarity%
26	Hexadecanoic acid, methyl ester	32.888	15.52	97
29	9-octadecenoic acid, methyl ester	36.301	21.98	99

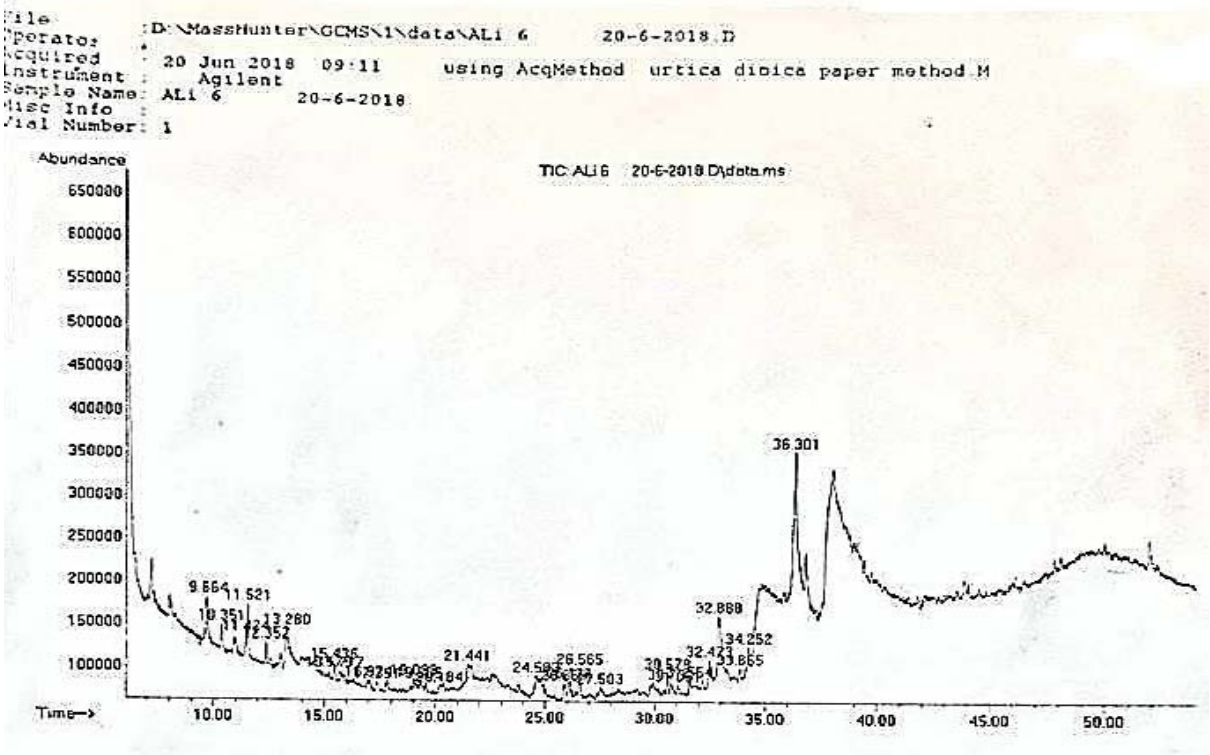

Figure 1. GC-Mass Chromatogram of AMRE.

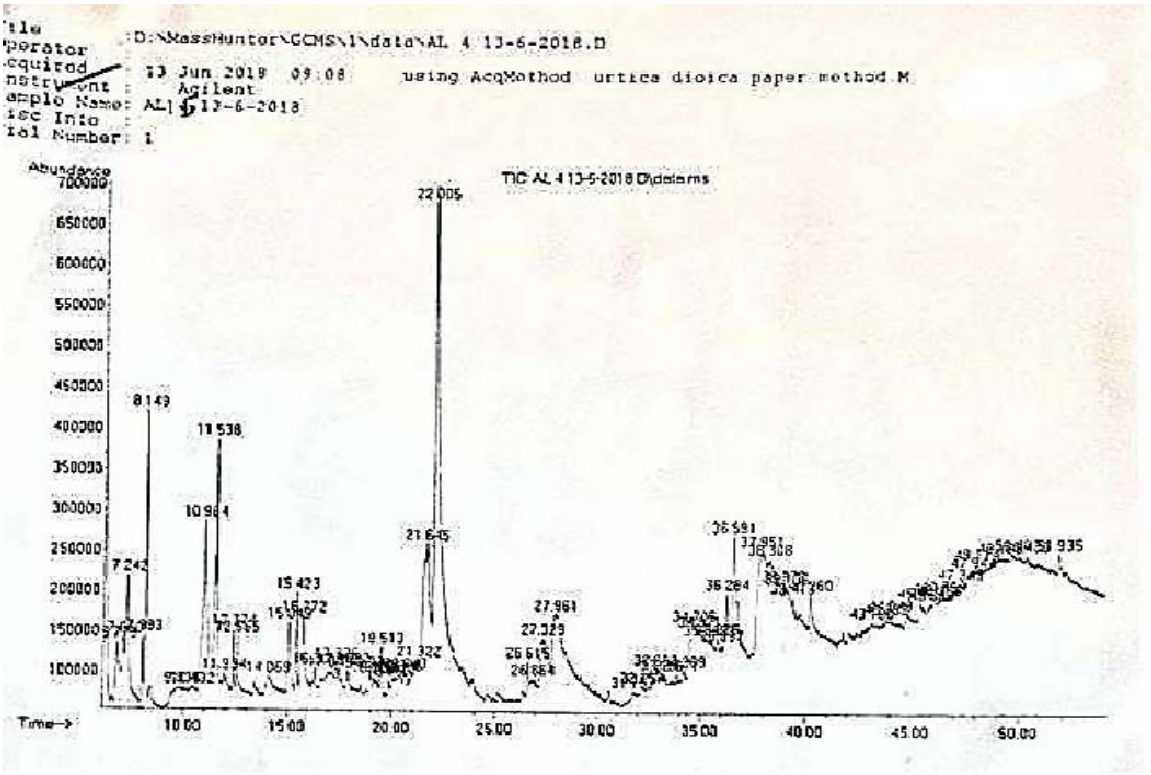

Figure 2. GC-MS Chromatogram of MMRE.

Table 4. Constitution of *Mirabilis* methanolic extract samples by GC-MS.

Peak No.	Compound	Retention time (R.T)	Area %	Similarity %
4	Cyclotetrasiloxane	7.982	0.47	72
5	Cyclotetrasiloxane, octamethyl-	8.146	2.57	90
7	Benzyl chloride	10.428	0.54	74
8	Arsenous acid, tris(trimethylsilyl) ester	10.960	4.94	78
12	Cyclopentasiloxane, decamethyl	12.565	0.60	87
14	Cyclopentasiloxane, octamethyl	15.050	1.16	87
31	Cyclododecane, acetic acid, trifluoro, undecyl ester, pentafluoropropionic acid	21.644	5.21	94
32	1-dodecanamine, N,N-dimethyl	22.001	15.71	86
35	Pentafluoropropionic acid, undecyl ester	27.329	2.36	94
49	Trans-13-octadecenoic acid, methyl ester	36.282	1.73	95
51	9-octadecenoic acid, E, cis-vaccenic acid, oleic acid	37.955	5.32	97
52	9-octadecenoic acid, E,-octadec-9-enoic acid, trans-13 octadecenoic acid	38.312	6.05	95
53	Oleic acid, 9,12-octadecienoic acid (Z,Z), 7-pentadecyne	38.970	0.80	95
54	9,12-octadecaienoic acid (Z,Z), cyclopentadecanone, 2 hydroxy E-11-hexadecenal	39.134	1.72	91
57	Cyclopropaneoctanal, 2-octyl-, 9-octadecenoic acid, (Z), 2,3-dihydroxypropyl ester	43.176	0.30	94

3.3. The DPPH free radical-scavenging activities of MMRE and AMRE

The scavenging action of both extracts, as determined by the DPPH test, is listed in Table 5. The results show that 250 mg ml^{-1} of MMRE has the highest scavenging activity of 96%. However, 250 mg ml^{-1} of AMRE had 89% scavenging activity. The antioxidant capability is assumed to be due to the occurrence of phytocomponent activities such as alkaloids, tannins, terpenes, saponins, and glycosides recognized in solvent extracts of *Mirabilis jalapa* [9]. The scavenging potential of rosy-flowered *Mirabilis J.* leaf in various diluents and the alcohol-soluble extractive rate show the occurrence of polar elements that have antioxidant ability [24, 28, 29].

Table 5. The scavenging activity of different concentrations of AMRE and MMRE.

Concentration (mg/ml)	AMRE (Inhibition) %	MMRE (Inhibition %)
50	34	45
100	54	55
150	78	65
200	80	84
250	89	96

4. Conclusion

In the present study, the presence of all the above-mentioned bioactive components in the extracts of *Mirabilis jalapa* L. is associated with an inhibitory effect against some pathogenic microorganisms, and the root extracts also showed *in vitro* antibacterial activity due to their content of glycosides, tannins, proteins, various phenolic compounds, alkaloids, and trace elements. However, if plant root extracts are to be used for food preservation or medical purposes, variations in chemical compounds can be used to differentiate among plants. The absence of chemical components such as phenols, flavonoids, alkaloids, fatty acids, etc., and their biological activity in human life should be highlighted. One of the most important aspects to be researched and considered is the genetic composition and its bioavailability.

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