Research Article

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Chemical compositions, antibacterial, antifungal and cytotoxic effects of *Alhagi mannifera* five extracts

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Abstract

Objectives: Plants were used as medicines thousands of years ago. Conventional medicine use is increasing and many of the currently used drugs are extracted from herbal sources. In Palestinian traditional medicine, the *Alhagi mannifera* plant is used for the treatment of cancer. Our study aimed to extract this plant using five solvent fractions, identifying their chemical compositions, and evaluating their antimicrobial and cytotoxic effects.

Methods: The successive technique was used to extract five solvent fractions of *A. mannifera*. While the spectral analysis was used to characterize quantitatively and qualitatively the chemical components of these extracts. The antimicrobial activity of plant extracts was evaluated against seven microbial strains using a broth micro-dilution assay. The cytotoxic activity was assessed using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay against cervical cancer cell line (HeLa).

Results: A total of 165 compounds were identified in *A. mannifera* different extracts. In the petroleum ether extract were found a total of 55 compounds. The major compounds were 2,5-cyclooctadien-1-ol (9.42%), 3-chloropropionic acid, heptyl ester (9.42%), carbonic acid, ethyl nonyl ester (9.42%) and chloroacetic acid. In methylene chloride extract a total of 11 compounds were found, and the major compounds were m-ainobenzenesulfonyl fluoride (14.35%), dodecane,2,6, 10-trimethyl- (14.35%) and propanoic acid,2,2-dimethyl-, 2-ethylexyl ester (14.35%). In chloroform extract, a total of 23 compounds were found. The major compounds were 5-ethyl-1-nonene (21.28%), and decanedioic acid, bis(2-ethylhexyl) ester (21.28%). In acetone extract were found a total of 47 compounds and the major compound was phenol,2, 4-bis(1,1-dimethylethyl)- (5.22%). In methanol extract a total of 29 compounds were found and the major compounds were 3-o-methyl-D-glucose (10.79%), myo-inositol, 2-c-methyl- (10.79%), myo-inositol, 4-c-methyl- (10.79%), and scyllo-inositol,1C-methyl- (10.79%). All extracts showed antimicrobial activity. However, the petroleum ether extract showed the most potent antimicrobial effect against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia*, MRSA, and *Candida albicans* with minimal inhibitory concentration (MIC) values of 1.25, 1.25, 6.25, 0.325, 6.25, and 1.56 µg/mL, respectively. De facto, chloroform extract followed by ether extract displayed potential cytotoxic activity with IC_{50} values of 0.2 and 1.2 mg/mL, respectively.

Conclusions: *A. mannifera* was found to contain a variety of phytochemicals and its chloroform extract showed a potent cytotoxic effect on HeLa cancer cells. In addition, petroleum ether showed potent antimicrobial agents and these extracts look promising as drug candidates. Further *in vivo* investigations should be conducted to provide the basis for developing new cancer and microbial infections treatments.

Keywords: Alhagi mannifera; antimicrobial; cytotoxicity; phytochemistry.

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Introduction

While conventional synthetic medicine is the leading treatment method for the majority of diseases around the

world, herbal medicine is still widely used, mostly, but not confined, in developing countries [1, 2]. The antimicrobial resistance problem has been attributed to the misuse and overuse of antibiotics, in addition to the lack of the discovery of new effective drugs by pharmaceutical companies due to the difficulties of antimicrobial drug registrations and the reductions in pharmaceutical investments [3, 4].

The International Agency for Research on Cancer published a worldwide statistic on cancer called GLOBOCAN and concluded that there are 18.1 million new cases with an estimated 9.6 million deaths caused by it [5]. The first line in the treatment of cancer is chemotherapy, and research focuses on how to improve this method, but it has many side effects, mainly neutropenia, stomatitis, mucositis, diarrhea, and emesis [6, 7]. For these side effects and other minor reasons, some people tend to refuse chemotherapy and use traditional and herbal medicine [8].

One of the herbals that used in the traditional treatment of cancer is *Alhagi mannifera* Jaub. & Spach (Fabaceae) which is an evergreen perennial herbaceous shrubby plant. Its branches grow over 0.6 m high. The gray-green branches are covered with long rigid twigs. Its leaves appear small, simple with a rounded tip at the base of every twig. Its flowers have a pink to maroon color and existing in pairs on each side of the twig with a thick straight dark brown pod with kidney-shaped, small, and brown seeds [9].

In Palestinian folk medicine, *A. mannifera* fruits are used as a decoction for the treatment of glandular cancer [10]. Therefore, the current study aims to identify the chemical components of *A. mannifera* five extracts using Gas chromatography-Mass spectroscopy (GC–MS) apparatus and to evaluate their antimicrobial and cytotoxic activity against seven microbial strains and cervical cancer cells line, respectively.

Material and methods

Plant materials

The leaves of the *A. mannifera* plant were collected from the Nablus region of Palestine in June 2019. The plant was characterized by the pharmacognosist Dr. Nidal Jaradat in the Pharmacognosy Laboratory/Pharmacy Department at An-Najah National University and herbarium was kept under the voucher specimen code: Pharm-PCT-65. The collected material was washed with distilled water three times and dried in the shade at ordinary temperature (25 ± 2 °C) and humidity (55 ± 5 RH). The dried materials were then ground coarsely and kept in glass jars for further use.

GC–MS characterization

The characterization of the chemical components of *A. mannifera* was achieved by Perkin Elmer Elite-5-MS fused silica capillary column (30 m × 0.25 mm, film thickness 0.25 µm), while Helium was used as a carrier gas at a standard flow rate of 1.1 mL/min. The temperature of the injector was adjusted at 250 °C with an initial temperature of 50 °C, an initial hold of 5 min, and a ramp of 4.0 °C/min to 280 °C. The total running time was 62.5 min and the solvent delay was from 0 to 4.0 min. MS scan time was from 4 to 62.5 min, covering a mass range of 50.00–300.00 *m*/*z*. The chemical ingredients of the extract were characterized by comparing their mass spectra with the reference spectra in the MS Data Centre of the National Institute of Standards and Technology, and by matching their Kovats and retention indices with values reported in the literature [11, 12].

Extraction of chemical constituents

Twenty grams of the dried powdered algal sample was successively extracted by the Soxhlet apparatus, according to the standard extraction method utilizing different organic solvents with analytical reagent quality obtained from Alfa Aesar (USA). These solvents were petroleum ether (40–60 °C), methylene chloride (39.6 °C), chloroform (61.15 °C), acetone (56 °C), and finally methanol (64.7 °C). To ensure the complete extraction process, exhaustive extraction was applied with each solvent for 10 h. Extracts of different organic solvents were collected separately into dry clean beakers, after that, they were recovered from the solvents by evaporating them in a rotary evaporator at 60 °C, The residue was then dried in desiccators for 1 h. The extracts were kept under vacuum desiccators until used for gas chromatography/mass spectrometry (GC–MS) analysis [13].

Antibacterial and antifungal activities

The antibacterial activity of the plant extracts was determined using seven strains of bacteria which were obtained from the American Type Culture Collection (ATCC) including Gram-positive types like *Enterococcus faecium* (ATCC 700221) and *Staphylococcus aureus* (ATCC 25923), and Gram-negative species such as *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883), and *Proteus vulgaris* (ATCC 8427). In addition, diagnostically confirmed Methicillin-Resistant *S. aureus* (MRSA) was utilized in our experimental work. The antifungal activity of the plant extracts was evaluated against the growth of *Candida albicans* (ATCC 90028). However, the antimicrobial activity of the plant of the five extracts used in this study was estimated using the broth microdilution method.

Each plant extract was dissolved in DMSO (Sigma, UK) to a concentration of 200 µg/mL. The produced solution was serially micro-diluted (two folds) 10 times in sterile Mueller-Hinton broth (Himedia, India). The dilution processes were performed under aseptic conditions in 96 well plates. In the micro-wells that were assigned to evaluate the antibacterial activity of the plant extracts, micro-well number 11 contained plant-free Mueller-Hinton broth, which was used as a positive control for microbial growth. On the other hand, micro-well number 12 contained plant-free and microbial-free Mueller-Hinton broth, this well was used as a negative control for microbial growth. Micro-wells numbers 1–11 were inoculated aseptically with the test microbes. The antimicrobial assessment was done in triplicates. All the inoculated plates were incubated at 35 °C. Regarding the *C. albicans*, the same method was used but using RPMI media instead of Mueller-Hinton broth. The incubation period lasted

for about 18–24 h for those plates inoculated with the test bacterial strains and for about 48 h for those plates inoculated with *C. albicans*. The lowest concentration of the plant extracts at which no visible microbial growth in that micro-well was observed, was considered as the minimal inhibitory concentration (MIC) of the examined samples. The antimicrobial activity was evaluated using known antimicrobial agents namely Ampicillin (Selleck Chemicals, USA) and Ciprofloxacin (Sigma-Aldrich, Germany) which were used as positive controls for antibacterial activity, and Fluconazole (Fisher Scientific, UK) was used as a positive control for antifungal activity [14].

Cell culture and cytotoxicity assay

HeLa cervical adenocarcinoma cancer cells with a passage number (93021013) were cultured in RPMI-1640 medium (Roswell Park Memorial Institute) (Biological Industries, Beit-Hemi), which was supplemented with 10% serum of fetal bovine, 1% Streptomycin/Penicillin antibiotics (BI, Mumbai, India), and 1% 2,5-Diamino-5-oxopentanoic acid (Sigma, UK). Cells were grown up in a humidified atmosphere with 5% CO_2 at 37 °C and seeded at 2.6×10^4 cells/well in a 96-well plate. After 48 h cells were incubated with various concentrations of the tested samples for 24 h. Cell viability was assessed by CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay according to the manufacturer's instructions (Promega Corporation, USA). Briefly, at the end of the treatment, 20 µL of MTS solution per 100 µL of media was added to each well and incubated at 37 °C for 2 h. Absorbance was measured at 490 nm.

Statistical analysis

All the data of cytotoxicity activity were presented as the average of triplicate analyses. The outcomes were presented as means ± standard deviation (SD). Statistical analysis was established employing GraphPad Prism software version 6.01 using t-test.

Results and discussion

According to the reports of the World Health Organization herbal medicine is the major source of primary health care for people living in developing countries [15]. Besides, natural herbal products are considered a major source of pharmaceutical preparations which were discovered mainly from traditionally prepared herbal extracts containing high contents of active ingredients [16].

Identification of the chemical components

The GC–MS apparatus was utilized to characterize qualitatively and quantitatively for the petroleum ether, methylene chloride, chloroform, acetone, and methanol extracts of *A. mannifera* leaves. Tables 1–5 and Figures S1–S5 depict the chemical ingredients of *A. mannifera* petroleum ether, methylene chloride, chloroform, acetone, and methanol extracts, respectively.

A total of 165 compounds were identified in different extracts. The petrolatum ether extract was found to have a total of 55 compounds, including 2,5-cyclooctadien-1-ol (9.42%), 3-chloropropionic acid, heptyl ester (9.42%), carbonic acid, ethyl nonyl ester (9.42%) and chloroacetic acid, heptyl ester (9.42%). In methylene chloride was found a total of 11 compounds, including m-ainobenzenesulfonyl fluoride (14.35%), dodecane,2,6,10-trimethyl- (14.35%) and propanoic acid,2,2-dimethyl-,2-ethylexyl ester (14.35%). The chloroform extract was found to have a total of 23 compounds including 5-ethyl-1-nonene (21.28%), decanedioic acid, bis(2-ethylhexyl)ester (21.28%), 1-heptacosanol (5.60%), dotriacontyl pentafluoropropionate (5.60%) and hexacosanol, acetate (5.60%). The acetone extract was found to have a total of 47 compounds, including: phenol,2,4-bis(1,1-dimethylethyl)- (5.22%), 5-eicosene (4.21%), 9-eicosene (4.21%) and dichloroacetic acid,4-hexadecyl ester (4.21%). In methanol extract was found a total of 29 compounds, including: 3-o-methyl-D-glucose (10.79%), myo-inositol, 2-c-methyl- (10.79%), myo-inositol, 4-c-methyl- (10.79%), scyllo-inositol,1C-methyl- (10.79%) and 3-penten-2-one, 4-methyl- (8.23%).

Antimicrobial effects

The antimicrobial activity of *A. mannifera* petroleum ether, methylene chloride, chloroform, acetone, and methanol extracts was determined using broth microdilution assay against selected infectious pathogens belonging to Gram-negative, Gram-positive and fungi strains. The results showed that *A. mannifera* petroleum ether, methylene chloride, chloroform, acetone, and methanol extracts have antimicrobial potentials as reported in Table 6. The greatest antibacterial activity was for *A. mannifera* petroleum ether extract. The extract showed activity against all the screened strains comparing with the MIC values of all positive controls. However, the petroleum ether extract showed the highest antimicrobial effects against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumonia*, MRSA, and *C. albicans* with MIC values of 1.25, 1.25, 6.25, 0.325, 6.25, and 1.56 µg/mL, respectively. While the methanol extract showed the highest inhibitory activity against *P. vulgaris* with a MIC value of 25 µg/mL. The importance of *Alhagi* plants in general and *A. mannifera* in particular for a variety of diseases treatment was shown in numerous studies such as the treatment of Shingles, ulcers, inflammations, headache, fever, bacterial infections, Urinary Tract Infections (UTI), kidney stones, hypertension, and cancer [17].

Table 1: The chemical constituents of *Alhagi mannifera* petroleum ether extract.

	Names of the compounds	RT	RI	Formula	%Area
1.	Pentadecanoic acid, 15-bromo-	46.85	708	$C_{15}H_{29}BrO_2$	0.63
2.	9,12,15-Octadecatrienoic acid, (z,z,z)-	46.275	900	$C_{18}H_{30}O_2$	5.61
3.	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	46.275	882	$C_{19}H_{32}O_3$	5.61
4.	Methyl 8,11,14-heptadecatrienoate	46.275	892	$C_{18}H_{30}O_2$	5.61
5.	Methyl 11,14,17-eicosatrienoate	46.275	877	$C_{21}H_{36}O_2$	5.61
6.	2-Chloroethyl linoleate	46.145	837	$C_{20}H_{35}ClO_2$	1.65
7.	Methyl 10,11-octadecadienoate	46.145	843	$C_{19}H_{34}O_2$	1.65
8.	Methyl 11,12-octadecadienoate	46.145	852	$C_{19}H_{34}O_2$	1.65
9.	Methyl 11,14-octadecadienoate	46.145	832	$C_{19}H_{34}O_2$	1.65
10	l-(+)-Ascorbic acid 2,6-dihexadecanoate	42.218	806	$C_{38}H_{68}O_8$	5.51
11.	n-Hexadecanoic acid	42.218	824	$C_{16}H_{32}O_2$	5.51
12.	Octadecanoic acid	42.218	788	$C_{18}H_{36}O_2$	5.51
13.	Tridecanoic acid	42.218	776	$CH_3(CH_2)_{11}COOH$	5.51
14.	1,2-Benzenedicarboxylic acid, butyl octyl ester	41.998	771	$C_{20}H_{30}O_4$	0.04
15.	1,2-Benzenedicarboxylic acid, diheptyl ester	41.998	757	$C_{22}H_{34}O_4$	0.04
16.	Phthalic acid, butyl nonyl ester	41.998	761	$C_{21}H_{32}O_4$	0.04
17.	Phthalic acid, isobutyl octyl ester	41.998	753	$C_{20}H_{30}O_4$	0.04
18.	2-Pentacosanone	39.182	820	$C_{25}H_{50}O$	0.57
19.	4-Methoxy-6-methyl-6,7-dihydro-4h-furo[3,2-c]pyran	39.182	816	$C_9H_{12}O_3$	0.57
20.	z-25-Tetratriaconten-2-one	39.182	767	$C_{34}H_{66}O$	0.57
21.	2-Pentacosanone4-methyloxy-6-methyl-6,7-dihydro-4h-furo[3,2-c]pyran	39.177	833	$C_{34}H_{62}O_4$	0.57
22.	1,16-Hexadecanediol	39.057	748	$C_{16}H_{34}O_2$	0.57
23.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	39.057	780	$C_{20}H_{40}O$	0.57
24.	6-Octen-1-ol,3,7-dimethyl-,propanoate	39.057	760	$C_{13}H_{24}O_2$	0.57
25.	Butanoic acid, 3-methyl-,3,7-dimethyl-6-octenyl ester	39.057	767	$C_{15}H_{28}O_2$	0.57
26.	z,z-6,28-Heptatriactontadien-2-one	39.057	806	$C_{37}H_{70}O$	0.57
27.	Dodecane,2,7,10-trimethyl-	26.472	670	$C_{15}H_{32}$	0.57
28.	Hentriacontane	26.472	760	$C_{31}H_{64}$	0.57
29.	Heptadecane, 2,6,10,15-tetramethyl-	26.472	713	$C_{21}H_{44}$	0.57
30.	Tritetracontane	26.472	685	$C_{43}H_{88}$	0.57
31.	Dodecane,1-fluoro-	22.221	748	$C_{12}H_{25}F$	0.18
32.	Sulfurouse acid, 2-ethylhexyl nonyl ester	22.221	703	$C_{17}H_{36}O_3S$	0.18
33.	Sulfurouse acid, decyl 2-ethylhexyl ester	22.221	707	$C_{18}H_{36}O_3S$	0.18
34.	Benzene,1,3-bis(1,1-dimethylethyl)-	21.25	713	$C_{14}H_{22}$	0.22
25.	p-Pentylacetophenone	21.25	643	$C_{13}H_{18}O$	0.22
36.	Acetic acid, cyano-, 2-ethylhexyl ester	15.903	605	$C_{11}H_{19}NO_2$	0.07
37.	Heptane, 1,1′-oxybis-	15.903	745	$C_{14}H_{30}O$	0.07
38.	1,1-Dimethylproppyl 2-ethylhexanoate	15.678	617	$C_{13}H_{26}O_2$	0.07
39.	Carbonic acid, isobutyl 2methylbutyl ester	15.678	652	$C_{10}H_2O_3$	0.07
40.	Oxalic acid, butyl 2-ethylhexyl ester	15.678	601	$C_{14}H_{26}O_4$	0.07
41.	Dodecane,4,6-dimethyl-	13.852	622	$C_{14}H_{30}$	0.13
42.	Nonane,4,5-dimethyl-	13.852	621	$C_{11}H_{24}$	0.13
43.	Propanoic acid,2,2-dimethyl-,2-ethylhexyl ester	13.852	631	$C_{13}H_{26}O_2$	0.13
44.	1,4-Dibromo-2-cyclohexylbutan	13.762	435	$C_{10}H_{18}Br_2$	0.13
45.	4-(2,2-dimethyl-6-methylenecyclohexyl)butanal	13.762	456	$C_{13}H_{22}O$	0.13
46.	4-Pentadecyne,15-chloro-	13.762	440	$C_{15}H_{27}Cl$	0.13
47.	6,10-Dodecandien-1-yn-3-ol,3,7,11-trilmethyl-	13.762	441	$C_{15}H_{24}O$	0.13
48.	3-Oxabicyclo[6.3.1]dodec-8-en-2-one	8.78	705	$C_{11}H_{16}O_2$	0.21
49.	Picolinyl 3,6,9,12,15-octadecapentaenoate	8.78	596	$C_{24}H_{31}NO_2$	0.21
50.	Picolinyl 6,9,12,15-octadecatetraenoate	8.78	587	$C_{24}H_{33}NO2$	0.21
51.	Picolinyl octadeca-6-yn-9,12,15-trienoate	8.78	585	$C_{13}H_{22}O$	0.21
52.	2,5-Cyclooctadien-1-ol	4.379	664	$C_8H_{12}O$	9.42
53.	3-Chloropropionic acid, heptyl ester	4.379	641	$C_{10}H_{19}ClO_2$	9.42
54.	Carbonic acid, ethyl nonyl ester	4.379	630	$C_{12}H_{24}O_3$	9.42
55.	Chloroacetic acid, heptyl ester	4.379	625	$C_9H_{17}ClO_2$	9.42
Total					100

Table 2: The chemical constituents of *Alhagi mannifera* methylene chloride extract.

	Names of the compounds	RT	RI	Formula	%Area
1.	m-Ainobenzenesulfonyl fluoride	21.26	607	$C_6H_5FO_2S$	14.35
2.	Dodecane,2,6,10-trimethyl-	19.544	636	$C_{15}H_{32}$	14.35
3.	Propanoic acid,2,2-dimethyl-,2-ethylexyl ester	19.544	657	$C_{13}H_{26}O_2$	14.35
4.	1r-.Alpha.-pinene	8.77	761	$C_{10}H_{16}$	8.75
5.	Bicyclo[3.1.0]hex-2-ene,2-methyl-5-(1-methylethyl)-	8.77	806	$C_{10}H_{16}$	8.75
6.	Bicyclo[3.1.0]hex-2-ene,4-methyl-1-(1-methylethyl)-	8.77	784	$C_{10}H_{16}$	8.75
7.	Bicyclo[3.1.1]hept-2-ene,3,6,6-trimethyl-	8.77	789	$C_{10}H_{16}$	8.75
8.	Cyclopropanecarboxylic acid,-2-(2-propynyl)methyl ester	4.304	569	$C_8H_{11}O_2$	5.48
9.	Methyl 2-pyridyl ketone 4-cyclohexylthiosemiCARBAZONE	4.304	518	$C_{14}H_{20}N_4S$	5.48
10	Phenyl crotyl sulfone	4.304	512	$C_{10}H_{12}O_2S$	5.48
11.	Salicyl alcohol	4.304	558	$C_7H_8O_2$	5.48
Total					100

Table 3: The chemical constituents of *Alhagi mannifera* chloroform extract.

	Names of the compounds	RT	RI	Formula	%Area
1.	5-Ethyl-1-nonene	59.285	738	$C_{11}H_{22}$	21.28
2.	Decanedioic acid, bis(2-ethylhexyl)ester	59.285	811	$C_{26}H_{50}O_4$	21.28
3.	1-Heptacosanol	57.509	743	$C_{27}H_{56}$	5.60
4.	Dotriacontyl pentafluoropropionate	57.509	743	$C_{35}H_{65}F_5O_2$	5.60
5.	Hexacosanol, acetate	57.509	777	$C_{28}H_{56}O_2$	5.60
6.	D-mannopentadecane-1,2,3,4,5-pentaol	44.719	667	$C_{15}H_{32}O_5$	1.43
7.	1,3-Dioxolane,2-Pentadecyl-	43.129	576	$C_{218}H_{36}O$	1.04
8.	3-Dodecanol, 3,7,11-trimethyl-	43.129	656	$C_{15}H_{32}O$	1.04
9.	3-Heptanol, 3,5-dimethyl-	43.129	582	$C_9H_{20}O$	1.04
10	3-Octanol, 3,6-dimethyl-	43.129	584	$C_{10}H_{22}O$	1.04
11.	.Alpha.-d-riboside, 1-o-dodecyl-	42.148	672	$C_{17}H_{34}O_5$	4.29
12.	Octadecanoic acid	42.148	644	$C_{18}H_{36}O_2$	4.29
13.	Tetradecanoic acid	42.148	638	$C_{14}H_{28}O_2$	4.29
14.	Tridecanoic acid	42.148	646	$CH_3(CH_2)_{11}COOH$	4.29
15.	6-Octen-1-ol, 3,7-dimethyl-,propanoate	39.072	727	$C_{13}H_{24}O_2$	1.99
16.	Butanoic acid, 3-methyl-,3,7-dimethyl-6-octenyl ester	39.072	719	$C_{15}H_{28}O_2$	1.99
17.	1,3-Dioxolane, 2-(1,1-dimethylethyl)-	37.622	633	$C_7H_{14}O_2$	1.85
18.	1,3-Dioxolane, 2-(1-methylpropyl)-	37.622	636	$C_7H_{14}O_2$	1.85
19.	4-Cyclohexene-1,2-dicarboxylic acid, 4-chloro-, bis(trimethylsily)ester	37.622	616	$C_{14}H_{25}ClO_4Si_2$	1.85
20.	Butane, 2-methoxy-2-methyl-	37.622	652	$C_6H_{14}O$	1.85
21.	Dodecane, 1-fluoro-	22.241	702	$C_{12}H_{25}F$	2.16
22.	Hentriacontane	22.241	736	$C_{31}H_{64}$	2.16
23.	Sulfurous acid, 2-ethylhexyl tridecyl ester	22.241	656	$C_{21}H_{44}O_3S$	2.16
Total					100

Furthermore, the benefit of antibiotic treatment was very limited, which requires the patient and his relatives to seek other alternative therapeutics. Subsequently, the experience in antibacterial effects of *A. mannifera* was introduced by using the water crude extract for the first time up to the best researcher knowledge in this study and others [18]. Consequently, although the medicines have been widely developed at the global level, herbal treatments are still used by many patients for both being cheap and safe treatments [19]. However, some reservations must be taken into account, which emphasizes the need to treat plants and herbals with more care and caution, as it has proven medical efficacy in parallel with its side effects.

Cytotoxic activity

Cytotoxic assays have gained increasing interest over recent years due to their great value in the biological

Table 4: The chemical constituents of *Alhagi mannifera* acetone extract.

	Names of the compounds	RT	RI	Formula	%Area
1.	2-Pyrazolin-5-one,4-acetyl-3-methyl-1-phenyl-	53.118	516	$C_{12}H_{12}N_2O_2$	0.95
2.	Cinnarizine	53.118	547	$C_{26}H_{28}N_2$	0.95
3.	Pyrazino[1,2-A][1,3]benzimidazol-8-amine, 1,2,3,4-tetrahydro-2-(2-phenylethyl)-	53.118	540	$C_{18}H_{19}N_4$	0.95
4.	5-Pyrimidinamine, n-methyl-2-(methylsulfonyl)-n-(phynylmethyl)-	52.663	822	$C_{13}H_{15}N_3O_2S$	2.44
5.	n-Benzyloxy-2-isopropoxycarbonylazetidine	52.663	796	$C_{14}H_{20}NO_2$	2.44
6.	Phenol, 2-benzyloxy-3,6-difluoro-	52.663	805	$C_{13}H_9F_2O_2$	2.44
7.	Trans-2,2′-bis(benzyloxy)stilbene	52.663	794	$C_{28}H_{24}O_2$	2.44
8.	Adipic acid, 4-heptyl isobutyl ester	48.731	639	$C_{17}H_{32}O_4$	1.46
9.	Adipic acid, butyl 2,4-dimethylpent-3-yl ester	48.731	644	$C_{17}H_{32}O_4$	1.46
10	Butyl citrate	48.731	729	$C_{18}H_{32}O_7$	1.46
11.	Chloroacetic acid, tetradecyl ester	47.561	702	$C_{16}H_{31}ClO_2$	2.80
12.	Cyclohexan,1,2,4,5-tetraethyl-,(1.alpha.,2.alpha.,4.alpha.,5.alpha.)-	47.561	713	$C_{14}H_{28}$	2.80
13.	Cyclohexane,1,2-dimethyl-3-pentyl-4-propyl-	47.561	706	$C_{16}H_{32}$	2.80
14.	Hexacosanol, acetate	47.561	719	$C_{28}H_{56}O_2$	2.80
15.	1-Heptacosanol	42.974	792	$C_{27}H_{56}O$	3.37
16.	9-Tricosene,(z)-	42.974	782	$C_{23}H_{46}$	3.37
17.	Acetic acid, chloro-,octadecyl ester	42.974	786	$C_{20}H_{39}ClO_2$	3.37
18.	3-n-Hexylthiolane,s,-Dioxide	42.959	840	$C_{10}H_{20}O_2S$	3.37
19.	cis-2-Methyl-4-N-butylthiane,s,s-dioxide	42.959	812	$C_{10}H_{20}O_2S$	3.37
20.	Trans-2-methyl-4-n-butylthiane,s,s-dioxide	42.959	816	$C_{10}H_{20}O_2S$	3.37
21.	l-(+)-Ascorbic acid 2,6-dihexadecanoate	42.168	735	$C_{38}H_{68}O_8$	0.11
22.	n-Hexadecanoic acid	42.168	761	$C_{16}H_{32}O_2$	0.11
23.	Tridecanoic acid	42.168	754	$C_{13}H_{26}O_2$	0.11
24.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	40.148	745	$C_{20}H_{40}O$	1.15
25.	e-10-Methyl-11-tetradecen-1-ol propionate	40.148	679	$C_{18}H_{34}O_2$	1.15
26.	Methyl 11,14-eicosadienoate	40.148	715	$C_{21}H_{38}O_2$	1.15
27.	z,z-6,28-Heptatriactontadien-2-one	40.148	711	$C_{37}H_{70}O$	1.15
28.	1,10-Hexadecanediol	39.692	652	$C_{16}H_{34}O_2$	0.78
29.	Cyclodecene,1,2-dimethyl-,(z)-	39.692	646	$C_{12}H_{22}$	0.78
30.	Cyclohexanemethanol,4-(1-methylethyl)-,cis-	39.692	643	$C_{10}H_{20}O$	0.78
31.	Cyclohexene,4-(4-ethylcyclohexyl)-1-pentyl-	39.692	642	$C_{19}H_{34}$	0.78
32.	5-Eicosene	32.4	755	$C_{20}H_{40}$	4.21
33.	9-Eicosene	32.4	754	$C_{20}H_{40}$	4.21
34.	Dichloroacetic acid,4-hexadecyl ester	32.4	754	$C_{18}H_{34}Cl_2O_2$	4.21
25.	Phenol,2,4-bis(1,1-dimethylethyl)-	29.894	879	$C_{22}H_{30}O$	5.22
36.	Benzene,1,3-bis(1,1-dimethylethyl)-	21.265	751	$C_{14}H_{22}$	1.03
37.	p-Pentylacetophenone	21.265	697	$C_{13}H_{18}O$	1.03
38.	Decane,3,8-dimethyl-	19.54	720	$C_{12}H_{26}$	3.33
39.	Dodecane,1-fluoro-	19.54	731	$C_{12}H_{25}F$	3.33
40.	Hentriacontane	19.54	769	$C_{31}H_{64}$	3.33
41.	Heptadecan,2,6,10,15-tetramethyl-	19.54	711	$C_{22}H_{44}$	3.33
42.	1R-.Alpha.-pinene	8.8	791	$C_{10}H_{16}$	2.21
43.	3-Carene	8.8	817	$C_{10}H_{16}$	2.21
44.	Bicyclo[3.1.1]hept-2-ene,3,6,6-trimethyl-	8.8	778	$C_{20}H_{32}$	2.21
45.	Bicyclo[3.1.1]hex-2-ene,2-methyl-5-(1-methylethyl)-	8.8	780	$C_{10}H_{16}$	2.21
46.	.Alpha.-pinene	8.775	793	$C_{10}H_{16}$	0.73
47.	1,3,6-Octatriene,3,7-dimethyl-,€	8.775	794	$C_{10}H_{16}$	0.73
Total					100

screening of insecticides, herbicides, and anticancer agents [20]. These assays proved to be reliable, quick, cheap, and reproducible. Various types of cytotoxic tests, such as luminometric, fluorometric, dye exclusion, and colorimetric assays, are used in the fields of pharmacology and toxicology [21]. The potential cytotoxicity of the *A. mannifera* towards the HeLa (cervical adenocarcinoma) cancer cell was evaluated using the MTS assay. As shown

Table 5: The chemical constituents of *Alhagi mannifera* methanol extract.

	Names of the compound	RT	RI	Formula	%Area
1.	n-Decanoic acid	46.81	552	$C_{10}H_{20}O_2$	0.45
2.	n-Hexadecanoic acid	42.148	762	$C_{16}H_{32}O_2$	2.93
3.	Octadecanoic acid	42.148	735	$C_{18}H_{36}O_2$	2.93
4.	Tetradecanoic acid	42.148	733	$C_{14}H_{28}O_2$	2.93
5.	Tridecanoic acid	42.148	743	$C_{13}H_{26}O_2$	2.93
6.	3-o-Methyl-D-glucose	35.651	692	$C_7H_{14}O_6$	10.79
7.	Myo-inositol, 2-c-methyl-	35.651	732	$C_7H_{14}O_6$	10.79
8.	Myo-inositol, 4-c-methyl-	35.651	749	$C_7H_{14}O_6$	10.79
9.	Scyllo-inositol,1C-methyl-	35.651	719	$C_7H_{14}O_6$	10.79
10	2,4,4-Trimethyl-1-pentanol	27.718	657	$C_8H_{18}O$	0.71
11.	Butoxyacetic acid	27.718	614	$C_6H_{12}O_3$	0.71
12.	Trifluoromethyl t-butyl disulfide	27.718	765	$C_5H_9F_3S$	0.71
13.	4-Hydroxy-2-methylacetophenone	23.491	805	$C_9H_{10}O_2$	0.71
14.	4-Hydroxy-3-methylacerophenone	23.491	798	$C_9H_{10}O_2$	0.71
15.	1,3-Dioxolane, 2-pentadecyl-	7.925	775	$C_{18}H_{36}O_2$	2.87
16.	3-Pentanol, 2,4-dimethyl-	7.925	689	$C_7H_{16}O$	2.87
17.	Butane, 2methoxy-2,3,3-trimethyl-	7.925	661	$C_8H_{18}O$	2.87
18.	n-Methoxy-n-trifluoroacetyl-1,1-dimethyl-2-carbomethylamine	7.925	766	$C_5H_{10}F_3NO_2$	2.87
19.	1,3,5-Trithiane, 2,4,6-trimethyl-	5.394	579	$C_6H_{12}S_3$	3.54
20.	Beta,'-dithiodilactic acid	5.394	474	$C_6H_{10}O_4S_4$	3.54
21.	Beta.-l-arabinopyranosie,methyl	5.394	555	$C_6H_{12}O_5$	3.54
22.	2-Hexanol, methyl ether	5.254	641	$C_7H_{16}O$	4.77
23.	4-Methyl-2-pentanol, methyl ether	5.254	665	$C_7H_{16}O$	1.75
24.	Pentadecanamide, 15-bromo-	5.254	597	$C_{15}H_{30}BrNO$	1.75
25.	Undecanamide, 11-bromo-	5.254	611	$C_{11}H_{22}BrNO$	1.75
26.	2,4-Azetidinedione, 3,3-diethyl-	4.118	554	$C_7H_{11}NO_2$	3.01
27.	3-Penten-2-one, 4-methyl-	4.118	591	$C_6H_{10}O$	8.23
28.	Furane, 2-methoxy-	4.118	549	$C_5H_6O_2$	3.01
29.	Glutamic acid, n-tiglyl-dimethyl ester	4.118	601	$C_{12}H_{19}NO_5$	3.01
Total					100

Table 6: Minimal inhibitory concentration (MIC) values (µg/mL) of *Alhagi mannifera* petroleum ether, methylene chloride, chloroform, acetone, and methanol extracts in µg/mL also for the Ampicillin, Ciprofloxacin, and Fluconazole.

Tested materials	Bacterial strains						Fungal strain
	S. aureus	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*P. vulgaris*	MRSA	*C. albicans*
Petroleum ether extract	1.25	1.25	6.25	0.325	25	6.25	1.56
Methylene chloride	25	50	25	12.5	25	25	6.25
Chloroform extract	12.5	6.25	25	25	12.5	50	50
Acetone extract	25	12.5	50	12.5	50	25	25
Methanol extract	50	12.5	12.5	6.25	3.12	50	12.5
Fluconazole	–	–	–	–	–	–	1.56
Ampicillin	3.12	3.12	–	1	18	0	–
Ciprofloxacin	0.78	1.56	3.12	0.125	15	12.5	–

in Figure 1, the chloroform extract displays potential cytotoxic activity with an IC_{50} value of 0.2 mg/mL followed by ether extract.

A cytotoxicity test was carried out for *Alhagi maurorum* on the human leukemia cell line (HL-60) and they found there's some inhibitory effect against the proliferation of this kind of cancer cells [22].

In a review study, was found that all species of *Alhagi* have a medical activity such as laxative, antibacterial, anti-inflammatory, antifungal, and others. For our species

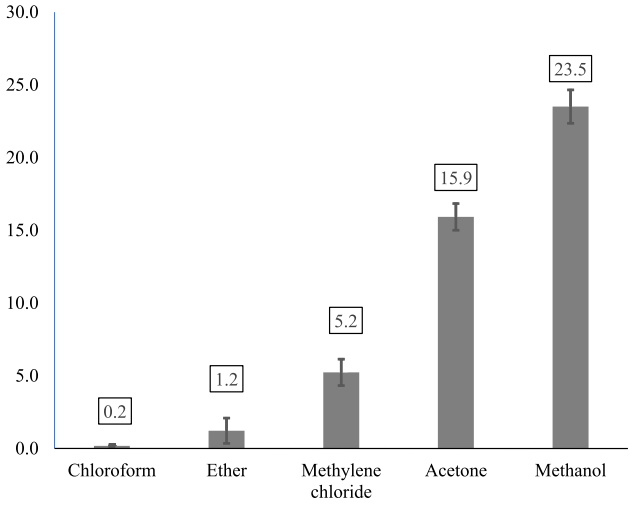

Figure 1: Cytotoxic activity IC_{50} values (mg/mL) of petroleum ether, methylene chloride, chloroform, acetone, and methanol extracts of *Alhagi mannifera*.

A. mannifera they found it can be used in liver disease, urinary tract infection, and gastrointestinal discomfort but in our study, we confirm the cytotoxicity and we need more studies to confirm other uses [23].

In a Saudi traditional study, they found that *A. mannifera* has dose-dependent cytotoxicity for ethanol, n-hexane, dichloromethane, and ethyl acetate extract. But the highest activity was for dichloromethane and ethyl acetate [24]. Also, the flavonol isorhamnetin from *A. mannifera* was found to have antiproliferative, necrotic, and apoptotic activity against human colon cancer cells [25].

Conclusion

The current results revealed the presence of many phytochemicals in the *A. mannifera* extracts. The results also showed that the chloroform and ether extracts have potential cytotoxic activity. Besides, the petroleum ether extract showed a potent antimicrobial effect against *S. aureus, E. coli, P. aeruginosa, K. pneumonia*, MRSA, and *C. albicans*. While the methanol extract showed the highest inhibitory activity against *P. vulgaris*. In fact, petroleum ether, chloroform, and ether extracts of the *A. mannifera* plant could be a promising candidate that can be used in future pharmaceutical formulation and a treatment strategy for cancer and microbial infectious diseases.

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