In Vitro Activity of Hexane and Ethanol Extracts of Camelthorn, *Alhagi maurorum* against Plant Pathogenic Fungi and Bacteria

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ABSTRACT- ***The effeciency of hexane and ethanol extracts of camelthorn, (Alhagi maurorum) was evaluated as antifungal agent, the activity of Alhagi maurorum was assessed using mycelial radial growth inhibition technique against five plant pathogenic fungi (Rhizocotonia solani , Fusariun moliniforme, Alternaria alternata, Botrytis cinerea and Sclerotia rolfsii). Eethanol extract of Alhagi maurorum displayed a very weak or no antifungal activity against the tested fungi. The antibacterial activity of camelthorn was also assessed against four plant pathogenic bacteria, Agrobacterium tumefaciens, Erwinia amylovolora, Pseudomonas solanacearum and Corynebacterium fascians, using agar dilution method. Hexane extracts of camelthorn showed a remarkable antibacterial activity with minimum inhibitory concentrations (MICs) 3000 mg/l, while ethanol extract exhibited a weak antibacterial activity. GC-MS analysis of ethanolic extract of Alhagi aurorum showed that the major compound have thirteen peaks indicating the presence of thirteen compounds.***

Keywords--- *Alhagi maurorum,* Plant pathogenic fungi**,** Plant pathogenic bacteria, GC- mass spectroscopy.

1. INTRODUCTION

Chemical control may be available to effectively and extensively reduce the effects of most fungal and bacterial diseases but field application of these chemical fungicides may not always be desirable. Excessive and improper use of these chemicals present a danger to the health of humans, animals, and the environment. Therefore, extensive search for biofungicides and biobactericides that are environmentally safe and easily biodegradable have been carried out during the last two decades [1], [2], [3], [4].

One source of potential new pesticides is natural products produced by plants. Some plants exert strong antimicrobial (antifungal and antibacterial) properties and could be conveniently used as a promising alternative source for presently problematic antifungal treatment in many areas with respect to their natural origin [5], [6], [7], [8], [9], [10].

Due to our climatic conditions, we have many valuable plants. *Alhagi maurorum* is one of these plants and it is a shrubby evergreen perennial herb, woody at the base , erect to ascending up to 60-100 cm high , very much branched with rigid spiny twigs about 1 inch long . The plant belongs to family leguminasae and native to North Africa, the Middle East and Southeast Europe [11]. The plant is a favorable food for camels, sometimes called Aqool, Shoakel-gamal, and camel thorn [12]. *Alhagi maurorum* is widely distributed and seems to have wide ecological amplitude, it recorded from Nile region, oasis, Mediterranean region, Eastern and Western Desert, Red sea Coast and Sinai, also in Saudi Arabia deserts [13], [14]. *Alhagi maurorum Boiss* is customarily used in folk medicine [15] as a remedy for rheumatic pains, bilharzias, liver disorders, and for various types of gastrointestinal discomfort [16].

Antifungal activity of *Alhagi* species extracts against plant pathogenic fungi was reported by many researchers [17], [18], [19], [9], [20], while the antibacterial activity of it against plant pathogenic bacteria was rarely reported.

Chemical investigation of *Alhagi* species revealed the presence of several constituents such as phenolic compounds [21]; [22], carbohydrate content [17], fatty acids and sterols [23]; [24], flavonoids [25]; [26]; [27], coumarins and alkaloids [28], proanthocyanidins [29], vitamins and lupeol [30]. Also, [22], [31] and [32] reported that Plant *Alhagi kirghisorum* have alkaloids, flavonoids, glycosides, steroids, terpenoids, resins and tannins.

Present investigation was undertaken to evaluate ethanol and hexane extracts of *Alhagi maurorum* with different concentrations for their antifungal activity against five plant pathogenic fungi (*Rhizocotonia solani,, Fusariun moliniforme, Alternaria alternata*, *Botrytis cinerea* and *Sclerotia rolfsii*). Also, for their antibacterial activity against four plant pathogenic bacteria *(Agrobacterium tumefaciens, Erwinia amylovolora, Pseudomonas solanacearum* and *Corynebacterium fascians),* besides identify the content of *Alhagi* extract by using GC-MS.

2. MATERIALS AND METHODS

2.1 Collection and identification of plant material

Samples of camelthorn (*Alhagi maurorum*) were collected and the fresh aerial parts were cleaned with water, air dried and coarsely powdered using a mortar and pestle, then further reduced to powder using electric blender.

2.2 Preparation of camelthorn (Alhagi maurorum) extracts

According to [10], the powdered plant material was extracted successively with ethanol and hexane, to obtain fractions with different polarities, using Soxhlet apparatus. After extraction the solvents were evaporated from crude extract by rotary evaporator. The dried extracts were weighed and then stored in air tight container and kept at 4°C until it was used for further analysis [33].

2.3 Fungi

The five fungi species used, *Rhizocotonia solani , Fusariun moliniforme, Alternaria alternata*, *Botrytis cinerea* and *Sclerotia rolfsii*, were obtained from the Fungicide Bioassay Laboratory, Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University. The fungi were maintained during the course the experiments on Potato Dextrose Agar (PDA) medium at 25 ºC.

2.4 Antifungal assay

The antifungal activity of hexane and ethanol extracts of camelthorn, (*Alhagi maurorum)* was tested using the radial growth technique method [34]. Appropriate volumes of the stock solutions of the plant extracts in dimethyl sulfoxide (DMSO) were added to Potato Dextrose Agar (PDA) to obtain a range of concentrations (5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0, 80.0 and 100.0g/L) immediately before pouring into the Petri dishes (9.0 cm in diameter) at 40-45ºC. Each concentration was tested in triplicate. Parallel controls were maintained with DMSO mixed with PDA. The discs of mycelial felt (0.5 cm diameter) of the plant pathogenic fungi, taken from 8-day-old cultures on PDA plates, were transferred aseptically to the centre of Petri dishes. The treatments were incubated at 25ºC in the dark. Colony growth diameter was measured after the fungal growth in the control treatments had completely covered the Petri dishes. Percentage of mycelial growth inhibition was calculated from the formula: Mycelial growth inhibition = [(DC-DT) /DC] × 100 [35], where DC and DT are average diameters of fungal colony of control and treatment, respectively. The concentration of extract that inhibiting the fungi mycelial growth by 50% (EC_{50}), was determined by a linear regression method [36].

2.5 Bacteria

Four bacteria species, *Agrobacterium tumefaciens, Erwinia amylovolora, Pseudomonas solanacearum* and *Corynebacterium fascians*, were used in this study. Microorganisms were provided by Microbiology Laboratory, Department of Plant Pathology, Alexandria University. The bacteria species were maintained on Nutrient Agar (NA: peptone 10 g, meat extract 5 g, sodium chloride 2.5 g and agar 10 g in 1000 ml distilled water at pH 6.5-6.6) medium.

2.6 Antibacterial assay

Agar dilution method was used, as recommended by European Society of Clinical Microbiology and Infectious Diseases [37], for determination of minimum inhibitory concentration (MIC) of hexane and ethanol extracts of camelthorn, (*Alhagi maurorum).* Extracts were dissolved in dimethylsulfoxide (DMSO). Appropriate volumes of the stock solutions were added to nutrient agar (NA)to obtain a range of concentrations 1, 2, 4, 6, 10 and 15 mg/l for hexane and ethanol extract and additional range was done (2.20, 2.40, 2.60, 2.80, 3.00, 3.20, 3.40, 3.60 and 3.80 g/l for hexane extract and 10.50, 11.00, 11.50, 12.00, 12.50, 13.00, 13.50, 14.00 and 14.50 g/l for ethanol extract) before pouring to Petri dishes. After solidifications, 6µl of bacterial cultures grown in a nutrient broth for 12 hours (approximately 10^8 CFU/ml) was spotted (three spots per each plate) using 2µl standard loop on the surface of agar. The inocula spots were allowed to dry before inverting the plates for incubation at 35ºC for 24h. Each plant extract was tested in triplicate. The

control was nutrient agar with a maximum volume of di-methy sulfoixde added to the treatments. The MIC was determined as lowest concentration of the extracts or the isolated compounds showing no visible bacterial growth in the agar plates.

2.7 Chemical analyses

Gas chromatography- mass spectrometry was used for Identification of crude extract. The GC–MS were performed in electronic impact ionization mode using a Aglient 6890 gas chromatograph equipped with an Aglient mass spectrometric detector, with a direct capillary interface and fused silica capillary column HP-5 ms (30 m x 0.32 mm x 0.25 µm film thickness). Samples were injected under the following conditions. Helium was used as carrier gas at approximately 1.0 ml/min., pulsed splitless mode. The solvent delay was 3 min. and the injection size was 1.0 µl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 e.v. scanning from m/z 50 to 500. The ion source temperature was 230°C. The electron multiplier voltage (EM voltage) was maintained 1250 v above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60°C (2 min) then elevated to 280°C at rate 8°C /min. the detector and injector temperature were set at 300 and 280°C, respectively. Wiley and Wiley Nist mass spectral data base was used in the identification of the separated peaks.

3. RESULTS AND DISCUSSION

3.1 Antifungal activity of ethanol and hexane extracts of camelthorn (Alhagi maurorum)

The antifungal activities of ethanol and hexane extracts of camelthorn (*Alhagi maurorum*) in terms of EC_{50} (The concentration of extract that inhibiting the fungi mycelial growth by 50%) are summarized in Table 1. Hexane extract of *Alhagi maurorum* showed the highest antifungal activity against *Rhizocotonia solani*, *Fusarium moliniforme* and *Alternaria alternate* with EC_{50} values of 25.20, 14.60 and 8.90 g L^{-1}, respectively. Hexane extract exhibited the strongest antifungal effect against *Botrytis cinerea and Sclerotia rolfsii* with EC_{50} values of 54.00 and 18.10 g L^{-1}. The same results were found by [9], who reported that hexane extract of *Alhagi maurorum* (Fabaceae) showed significant activity against *solani*, *Fusarium oxysporum*, *Aspergillus flavus*, *Alternaria alternate*, *Rhizoctnia solani*, *Pythium ultimum* and *Bipolaris oryzae*,

Alternaria alternate was most sensitive than the other tested fungi to hexane extract of camelthorn, while *Fusarium moliniforme* was the most sensitive one to ethanol extract. On the other hand, *Botrytis cinerea* was the most tolerant one to the two solvents of extract. Our results in this point were supported by the results of [18], who found that the ethanolic extract of thorn (*Alhagi maurorum Medic*.) had different degrees of antifungal activity against *Alternaria alternata*, *Fusarium oxysporum*, *Phoma destructiva*, *Rhizoctonia solani*, and *Sclerotium rolfsii.* Concerning to the sensitivity of *Fusarium sp* to ethanol extract, the same conclusion was reported by [9] and [20].

Table 1. Fungicidal activity of hexane and ethanol extracts of camelthorn (*Alhagi mmaurorum*) against five plant pathogenic fungi

Fungus	EC_{50} (g L^{-1})	95% Confidence limits		Slope
		Upper	Lower	
		Hexane extract		
Rhizocotonia solani	25.20	27.70	23.00	3.06
Fusarium moliniforme	14.60	16.60	12.80	1.94
Alternaria alternate	8.90	11.20	6.60	1.22
Botrytis cinerea	94.90	164.30	61.10	1.49
Sclerotia rolfsii	24.80	27.10	22.70	3.37
		Ethanol extract		
Rhizocotonia solani	36.80	39.60	34.20	5.32
Fusarium moliniforme	17.50	19.50	15.70	2.38
Alternaria alternate	26.80	44.80	18.60	0.69
Botrytis cinerea	54.00	69.00	43.40	2.17
Sclerotia rolfsii	18.10	20.20	16.20	3.32

3.2 Antibacterial activity of ethanol and hexane extracts of camelthorn (Alhagi maurorum)

Minimum inhibitory concentrations (MICs) values of the ethanol and hexane extracts of *Alhagi maurorum* were evaluated against four plant pathogenic bacteria using agar dilution assay are shown in Table 2. The result indicates that hexane extract of camelthorn exhibits a good antibacterial potency with MIC values ranged between 3.00 and 3.20 g L-1 against all the tested bacteria species. In contrary, we observed no noticeable antibacterial activity difference when ethanol extract of *Alhagi maurorum* used against all the tested bacteria species with MIC values ranged between 13.50 and 14.50 g L^{-1}. Our results were inagreement with [38] who reported that the hexane extract of *Alhagi graecorum* revealed moderate activity against the Gram-positive bacteria *S. pneumonia* and the Gram-negative bacteria *E. Coli.* An opposite results were obtained by [19], who reported that the ethanol extract of *Alhagi maurorum* showed inhibitory effect concentration against Fourteen bacterial strains; Gram-negative bacteria, *Acinitobacter baumanni*, *Escherichia coli*, *Moraxella lacunata*, *Proteus merabiles*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, Gram-positive bacteria, *Bacillus subtilis*, *Micrococcus kristinae*, *Micrococcus luteus*, *Sarcina ventricull*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Stroptococcus byogenes.*Also, methanol extract of *Alhagi camelorum* flowers had remarkable antibacterial activity against *Escherichia coli* (Gram-negative bacterium) and *Staphylococcus aureus* (Gram-positive coccal bacterium), [17].

Table 2. Minimum inhibitory concentrations (MICs, g L^{-1}) of hexane and ethanol extracts of camelthorn (*Alhagi maurorum*) using agar dilution method.

Bacteria	MICs (g L^{-1})	
	Hexane extract	Ethanol xtract
Agrobacterium tumefaciens	3.00	14.50
Erwinia. Amylovolora	3.00	13.50
Pseudomonas solanacearum	3.00	14.00
Corynebacterium fascians	3.20	13.50

3.3 GC analysis of ethanolic extracts of camelthorn (Alhagi maurorum)

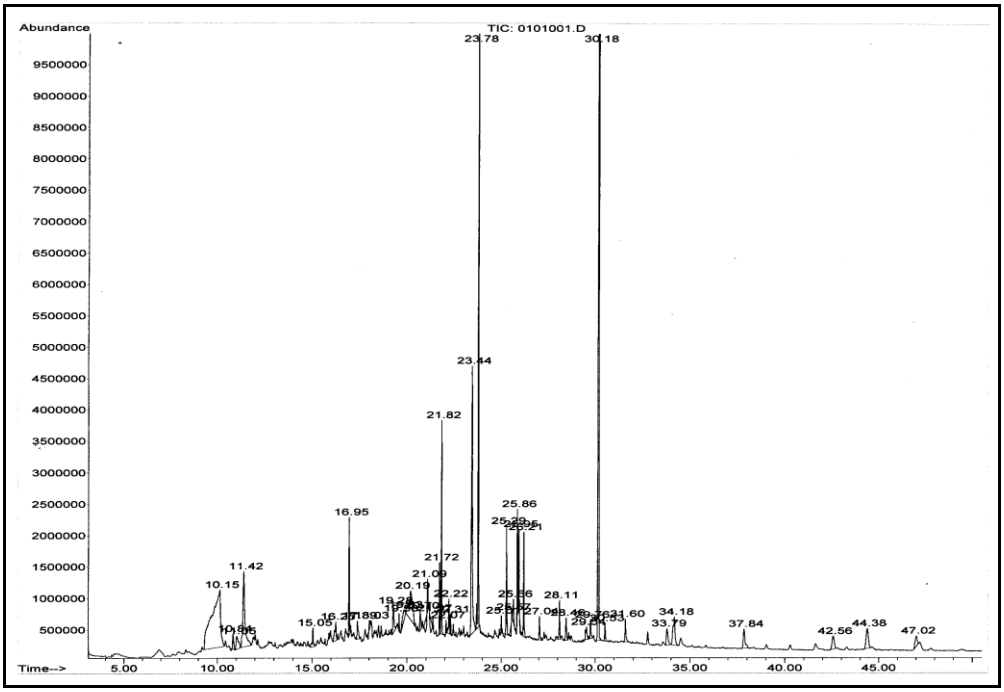
The Spectra of compound were matched with Wiley library. Their structures were identified by percentage similarity values. GC-MS analysis of ethanolic extract of *Alhagi maurorum* showed that the major compound have thirteen peaks indicating the presence of thirteen compounds shown in table (3). Antifungal compounds from *Alhagi maurorum* extract are form a wide range of chemical classes, including phenolic compounds [22]; [21], carbohydrate content [17], fatty acids and sterols [24], flavonoids [27], alkaloids and tannins [22] and vitamins and lupeol [30]. GC-MS analysis of ethanolic extract of camelthorn showed 1,2-benzenedicarboxylic acid bis (2-ethylhexyl) ester, 2-2-ethylhexyl ester, Hexadecanoic acid-ethyl ester and n-hexadecanoic acid with percentages of total 30.75%, 13.74%, 10.92% and 9.97%, respectively.

Table 3. GC-MS analysis of the major compound from camelthorn (*Alhagi maurorum*) extract.

No.	R.T	Chemical name	% total
1	10.14	2-2-ethylhexyl ester	13.74
2	10.846	Diethyl succinate	0.42
3	11.05	Methylacetophenone	0.85
4	11.42	1,4-Dihydronaphthalene	4.28
5	15.05	Trimethyi-1,6,7,7a-tetrahydrocyclopenta [c] pyran-l-yl) ethanone	0.53
6	16.25	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	0.73
7	16.95	2,6-bis(1,1-dimethylethyl)-4-methylphenol	1.92
8	17.39	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- (R)-	0.56
9	18.03	3,5-Heptanedione, 2,2,6,6-tetramethyl-Pyridine-2-thiol-1 –oxide	0.12
10	19.28	3,3,7-Trimethyl-2,9-dioxatric	0.87

No.	R.T	Chemical name	% total
11	19.57	3-ethyl-6-dimethylamino methylene	0.26
12	19.83	6-methoxy-6-methyl -2-cyclohexenone	0.73
13	20.19	2-methyl -1-thia-cyclopentane	2.98
14	20.69	Tetradecanoic acid	0.51
15	21.09	Undecanoic acid ethyl ester	0.26
16	21.72	Neophytadiene	1.16
17	21.83	2-pentadecanone 6, 10, 14-trimethyl	3.23
18	22.07	14-ethylene-14-pentadecane	0.15
19	22.23	1 2-benzenedicarboxylic acid	0.68
20	22.31	1,4-Eicosadiene	0.28
21	23.44	n-hexadecanoic acid	9.97
22	23.78	Hexadecanoic acid, ethyl ester	10.92
23	25.02	tetradecanoic acid ethyl ester	0.24
24	25.29	3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol	1.80
25	25.57	9,12-Octadecadienoic acid	0.26
26	25.66	9, 12, 15-octadecatrienoic acid methyl ester	0.56
27	25.87	Linoleic acid ethyl ester	2.89
28	26.21	Octadecanoic acid, ethyl ester	1.80
29	27.04	Tributyl acetylcitrate	0.49
30	28.11	cis-4-hydroxy-3-methylbutanoic acid lactone	0.84
31	28.46	Stearic acid ethyl ester	0.63
32	29.54	Eicosane	0.35
33	30.18	1,2-benzenedicarboxylic acid bis (2-ethylhexyl) ester	30.75
34	30.52	Docosanoic acid ethyl ester	0.38
35	31.60	n-Heptacosane	0.56
36	33.79	1 -Benzyl-4,6-dimethoxy-2,3-diphenyl indole	0.75
37	34.18	Octadecane 9-ethyl-n-heptyl	2.38
38	42.56	stigmasta-5 22-dien-3-ol	0.18

Figure 1. GC-MS Analysis of the major compound from camelthorn (*Alhagi maurorum*) extract



In conclusion, we can say that our results may spot on the camel thorn extract which is one of the most promising plants in the controlling of plant fungal and bacterial diseases as it contains compounds which have antifungal and antibacterial activity.

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