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Antioxidant, Antibacterial and GC/MS volatiles profiling of *Alhagi graecorum* Boiss

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ABSTRACT

Alhagi graecorum methanolic crude extract and its derived fractions (methylene chloride (mc), n-hexane (nh), n-butanol , and ethyl acetate finally the residual aqueous fraction) ;all were examined for their potential antioxidant activity performing the Superoxide dismutase like activity besides DPPH (α-diphenyl-β-picrylhydrazyl) assays. Moreover, the antibacterial property towards different pathogenic microbial Gram-positive *;(Staphylococcus aureus,(BS) Bacillus subtilis)* in addition to Gram-negative strains*(Proteus volgaris(PV),* and *Escherichia coli(EC)).* Identification of the secondary volatile metabolites of both *Alhagi graecorum* hexane and methylene chloride fractions was by Gas Chromatography/Mass Spectra (GC-MS) method. The identified extracted volatile constituents could be mainly categorized to two main classes, oxygenated acetogenins (fat derivatives) and oxygenated terpenes. Twelve volatile constituents were characterized in the hexane fraction, the most dominant compounds were methyl oleate (36.07%), methyl hexadecanoate (17.32%), methyl 11E,14E-octadecadienoate (13.32%). Of the eighteen constituents identified in the methylene chloride fraction, the most dominant were 4-(3-hydroxy-2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-but-3-en-2-one (5.51%), 2-dodecen-1-ylsuccinic anhydride (7.24%) and (–)-loliolide (19.73%).

Key Words:
Alhagi graecorum, leguminasae, GC/MS analysis, Antioxidant, Antibacterial

1. INTRODUCTION

Alhagi graecorum Boiss (Leguminasae) is a shrubby evergreen perennial herb native to North Africa commonly called Shouk Aljemal, Al-Aqool, and Camel thorn. In Egypt, it is widely distributed with a wide ecological amplitude which recorded from Red Sea coast and Sinai, Eastern and Western Desert, Mediterranean region and Nile region [1].

Numerous studies identified different categories of secondary metabolites from *A. graecorum* including phenolics, flavonoids, tannins, steroids, alkaloids, terpenoids and resins [2-4].

Traditionally, *Alhagi graecorum* Bioss species is used as a general tonic, anthelmintic, blood purifier, and to treat constipation, wounds, and arthritis. It is also used for dysentery, upper respiratory system problems, uterus problems and hemorrhoids. The roots are used for liver disorders, rheumatic pains, urinary tract infection hemorrhoids [2,5] and Jaundice [6]. Pharmacological studies of *A. graecorum* extracts or its purified phytochemicals revealed its cytotoxic, antioxidant, antimicrobial, hepatoprotective and antiproliferative activities [3, 7].

our current experiment aimed to consider *Alhagi graecorum* Boiss as a possible therapy by the usage of bioactive fractions through studying the antioxidant and antibacterial capacity of the extracted fractions, along with studying GC/MS volatiles profiling.

2. Experimental

2.1. General

Gas Chromatography (GC-MS) examination of the volatile extracted fractions were performed on a Varian GC(VGC) interfaced to a Finnegan SSQ 7000Mass selective Detector (SMD) having a data system identified as ICIS V2.0 for (MS) mass spectra identification of the Gas Chromatography constituents. The internal temperature of the oven, at isothermal condition, had a program for approximately 3 min. from about 50°C, then and there isothermally heating by 7°C /min. to 250°C and finally for nearly 10 min., at 250°C. The net injected volume was about 0.5 µl ,at this time the injector temperature was at 200°C and the temperature of ion source and transition-line were 150°C and 250°C, correspondingly. The column utilized was a DB-5 (J&W Scientific, Folosm, CA) capillary fused cross-linked silica column with (1/4 mm. interior diameter, nearly 30 m. length) , covered by polydimethyl-siloxane (0.5 µm. film thick).In order to avoid the solvent plead ,the mass spectrometer(ms) had a postponement for 3 minutes and then scanned from approximately 50 m/z to about 300 m/z. At about 70 eV , the ionization energy was previously set (The Agriculture Research Center in Dokki , Cairo governorate).

2.2. Solvents and chemicals

Hexane, methylene chloride, butanol, methanol, and anhydrous sodium sulphate were purchased from ADWIC Company.

2.3. Plant material

Alhagi graecorum Boiss was collected from Baltiem, Kafr Al sheikh in september 2018. Professor. Dr. Ibrahim Mashaly, herbarium, Botany Department, Science Faculty, University of Mansoura confirmed the identification,.

2.4. Extraction process

Dried and powdered airborne parts of *Alhagi graecorum* (1 kg) was soaked in methanol (3 x 2 L) then filtrated and evaporated using rotary evaporator to its 1/3 volume. Exhaustive liquid–liquid abstraction by n- butanol, (ea)eathyle acetate , (mc)methylene chloride ,n-hexane to yield 4.1 g, 1.32 g , 4.0 g 14.92 g fractions, in order. The final aqueous layer was left to dry and afforded a yield of 47.99 g.

The major and volatile hexane and methylene chloride fractions were defatted using cold methanol and subjected to GC/MS analysis to study their profile.

2.5. Biological activities assays

In vitro antibacterial effectiveness was evaluated using conventional Broth dilution method [8]. The possible antioxidant activity was estimated by DPPH free radical scavenging method [9] and SOD-like activity assay [10].

3. RESULTS AND DISCUSSION

The methanolic crude extract of *A. graecorum* aerial parts were fractionated to n-hexane, n-butanol, ethyl acetate(ea), methylene chloride, finally the remaining aqueous fraction ,all, were tested for possessing antibacterial and anti-oxidant properties.

The GC/MS technique was used in the exact identification of the extracted volatile phytochemicals in the hexane as well as methylene chloride(mc) fractions, as they were predominant and volatile. Application of GC/MS to hexane fraction (Table 1, Fig. 1) has led to the characterization and quantification of twelve volatile constituents. The main components were methyl oleate (36.07%), methyl hexadecanoate (17.32%) and methyl 11E,14E-octadecadienoate (13.32%). While for the methylene chloride fraction (Table 1, Fig. 1) eighteen volatile constituents were cautiously identified constructed on their relative mass spectra (MS) analysis then matched with constituents in NIST library, plus accurate comparison with other published spectroscopic data . Among the identified elments, (–)-loliolide (19.73%), 2-dodecen-1-ylsuccinic anhydride (7.24%), at last 4-(3-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-but-3-en-2-one (5.51%) were the most predominant.

The GC/MS analysis has shown that the both fractions are mainly composed of oxygenated acetogenins and oxygenated terpenes. Among the most predominant identified volatile constituents was methyl hexadecanoate (17.32%) which has antioxidant [11], antimicrobial activity [12] and antifungal activity [13]. Also, the monoterpenoid lactone (–)-loliolide which represented (19.73%) of methylene chloride fraction has antioxidant, anti-fungal, antibacterial, and anti-cancer activities [14].

Table 1: The volatile chemical composition of hexane and methylene chloride fractions of *Alhagi graecorum*

No.	Compound name	RT	Area %		M.F	M.Wt	Ref
			Hexane	CH_2Cl_2			
Acetogenins (Fat derivatives)							
1	(Z)-Hexadec-7-enal **(1)**	15.05		1.59	$C_{16}H_{30}O$	238	15
2	2-(Hexadecyloxy)ethanol **(2)**	15.11		2.22	$C_{18}H_{38}O_2$	286	16
3	(8Z)-7-Methyl-8-tetradecenyl acetate **(3)**	17.73		2.03	$C_{17}H_{32}O_2$	268	17
4	10-Methyl-8-tetradecen-1-ol acetate **(4)**	18.89		3.69	$C_{17}H_{32}O_2$	268	18
5	2-Dodecen-1-ylsuccinic anhydride **(5)**	19.12		7.24	$C_{16}H_{26}O_3$	266	19
6	Hexadecanoic acid, methyl ester **(6)**	21.53	17.32		$C_{17}H_{34}O_2$	270	20
7	Methyl (13Z,16Z)-docosadienoate **(7)**	21.81		2.67	$C_{23}H_{42}O_2$	350	21
8	Methyl oleate **(8)**	24.15	36.07		$C_{19}H_{36}O_2$	296	22
9	Methyl 11,14-octadecadienoate **(9)**	24.38	13.32		$C_{19}H_{34}O_2$	294	23

10	Methyl linolenate **(10)**	24.80	2.72		$C_{19}H_{32}O_2$	292	24
11	Ethyl Oleate **(11)**	24.93	2.85		$C_{20}H_{38}O_2$	310	25
12	Butyl 9,12-octadecadienoate **(12)**	25.16	0.80		$C_{22}H_{40}O_2$	336	26
13	Pentylelaidate **(13)**	27.18	1.65		$C_{23}H_{44}O_2$	352	27
14	(6Z)-5-Methyl-6-henicosen-11-one **(14)**	27.97		3.99	$C_{22}H_{42}O$	322	28
15	cis-5,8,11,14,17-Eicosapentaenoic acid **(15)**	29.43	1.79		$C_{20}H_{30}O_2$	302	29, 30
Oxygenated Monoterpenes							
16	5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-one **(16)**	20.18		4.26	$C_{14}H_{20}O_3$	236	31
17	4-(3-Hydroxy-2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-but-3-en-2-one **(17)**	20.77		5.51	$C_{13}H_{20}O_3$	224	32
18	(–)-Loliolide **(18)**	23.65		19.73	$C_{11}H_{16}O_3$	196	33
Oxygenated sesquiterpenes							
19	2-Methyl-9-(prop-1-en-3-ol-2-yl)bicyclo[4.4.0]dec-2-en-4-ol **(19)**	18.90	0.93		$C_{15}H_{24}O_2$	236	34
20	1,8-Dimethyl-8,9-epoxy-4-isopropylspiro[4.5]decan-7-one **(20)**	19.47		4.02	$C_{15}H_{24}O_2$	236	35
21	(+)-Drimane-8,11-diol **(21)**	19.70		1.53	$C_{15}H_{28}O_2$	240	36
22	Corymbolone **(22)**	19.85		0.70	$C_{15}H_{24}O_2$	236	37
23	Lactaropallidin **(23)**	19.91		2.29	$C_{15}H_{24}O_3$	252	38
24	(+)-(S)-ar-Turmerone **(24)**	19.95	1.23		$C_{15}H_{20}O$	216	39
25	Uvidin A **(25)**	20.99		1.23	$C_{15}H_{24}O_3$	252	40
26	Jaeschkeanadiol p-methoxybenzoate **(26)**	28.87	1.82		$C_{23}H_{32}O_4$	372	41
27	Isochiapin B **(27)**	32.18		0.87	$C_{19}H_{22}O_6$	346	42
Oxygenated Diterpenes							
28	Villosin **(28)**	31.22		1.89	$C_{20}H_{28}O_2$	300	43
29	2-[4-Methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde **(29)**	34.26		3.23	$C_{23}H_{32}O$	324	44
30	[6,11,14-Trihydroxy-4-(hydroxymethyl)-8,12,15,15-tetramethyl-7-oxo-3-oxapentacyclo[9.5.0.02,4.06,10.014,16]hexadec-8-en-13-yl] acetate **(30)**	35.19	1.51		$C_{22}H_{30}O_8$	422	

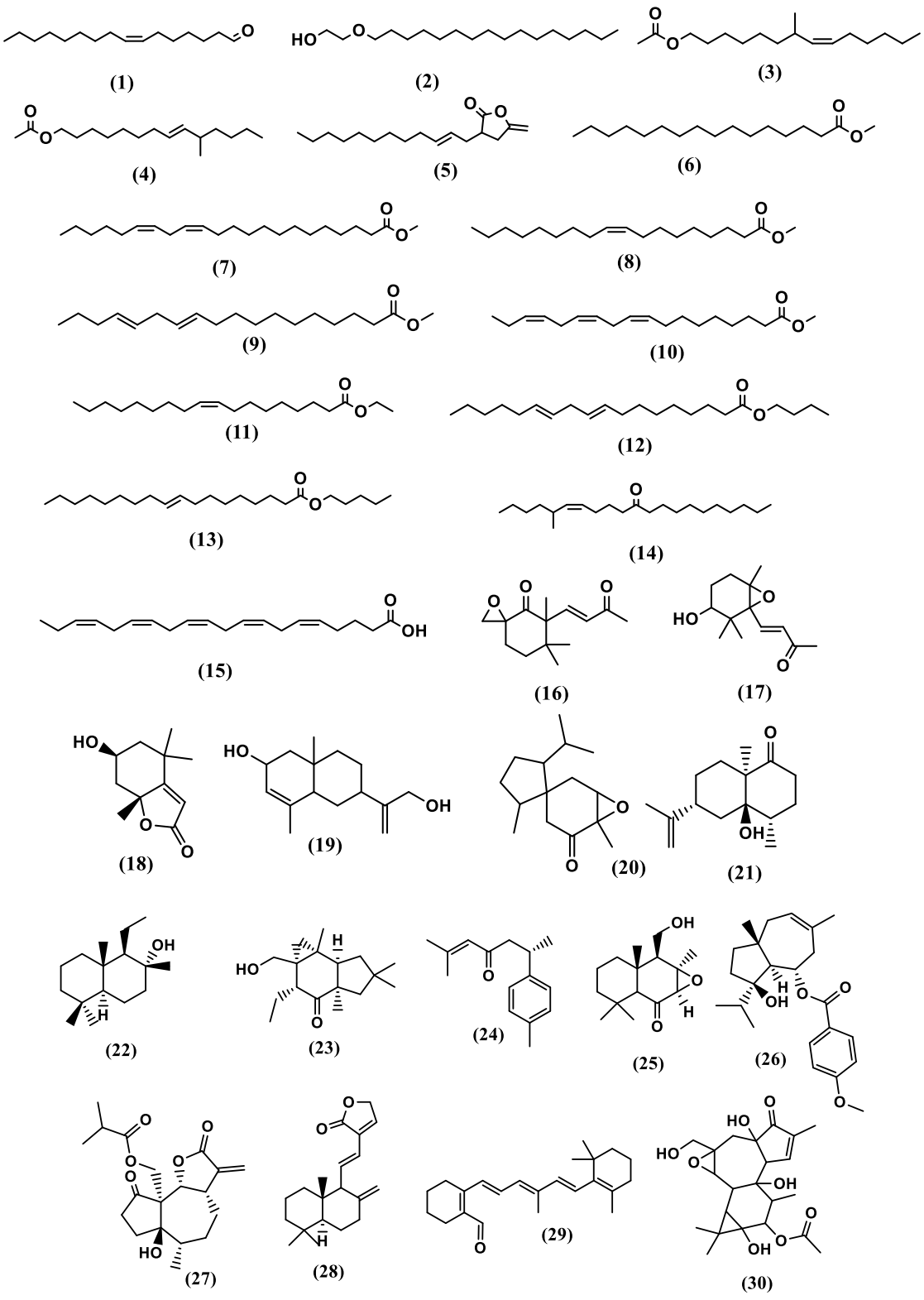

Figure 1: Structures of the identified *A. graecorum* volatile compounds

3.1Antibacterial activity

One of the most broadly used method to inspect plant extracts antibacterial activity is agar diffusion methods [45-46]. The antibacterial activities of *A. graecorum* methanolic extract and its

derived fractions were tested by the common disc diffusion technique towards two strains of Gram-positive bacteria ;*Staphylococcus aureus(SA), Bacillus subtilis (BS)* also two Gram-negative strains; *Escherichia coli(EC),Proteus volgaris(PV),*. The results (Figure 2) showed maximum growth inhibition for ethyl acetate fraction, crude methanol extract and hexane fraction against Gram-positive bacteria, while against Gram-negative strains hadn't any marked antibacterial potential. Generally, the antibacterial activity of the examined fractions was obviously more powerful towards Gram-positive bacterial strains growth in comparison with that of bacterial strains of Gram-negative . Actually, Gram-positive bacteria stereotypically has less resistance to antimicrobial agents than Gram-negative bacteria, and this resistance difference may be due to the existence of an outer-membrane permeability barrier in Gram-negative , that restricts entree of the antimicrobial compounds to their targeted organelles in the typical bacteria cell [47].

The observed findings approved with those established by an earlier report investigating antimicrobial activity of Alhagi graecorum of Saudi Arabia [48]. Ethyl acetate extract of *A. graecorum* showed the maximum antimicrobial activity followed by dichloromethane extract, crude alcoholic extract, and n-hexane extract. Several species of *Alhagi* were reported to contain high concentrations of flavonoids glycosides which well-known by their ability to complex with bacterial cell walls as well as with extracellular and soluble protein [48].

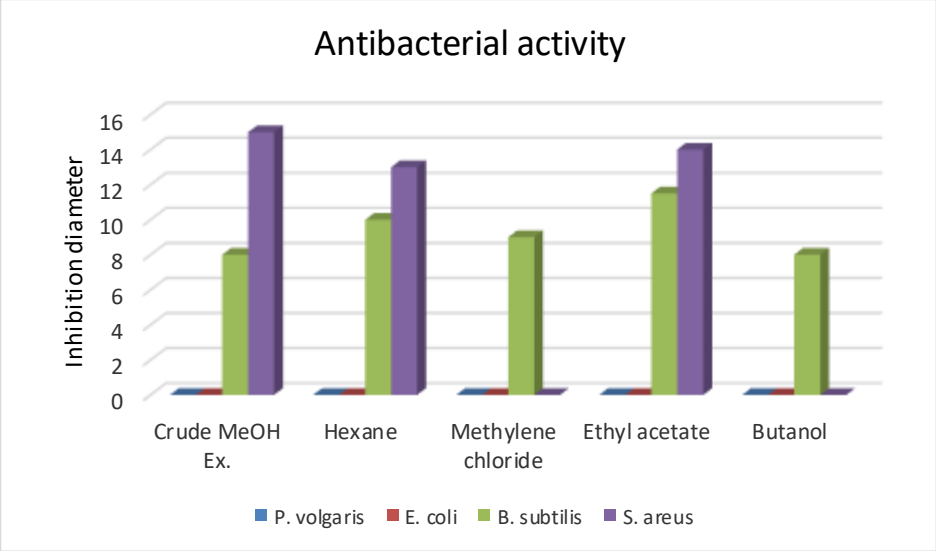

Figure 2: The antibacterial activity of *A. graecorum* extracts

3.2Antioxidant activity

Among many methods performed to assess the antioxidant ability of some plants, the precise super oxide dismutase (SOD)-like activity and (DPPH)1,1-diphenyl-2-picrylhydrazyl free radical scavenging assay were utilized to define the potential antioxidant capacity of the *A. graecorum* derived extracts. These both different methods, with essentially different approaches, are acknowledged for

high sensitivity and effective perfect results. The whole derived extracts exhibited anti-oxidant capacity (Figure 3). Nevertheless, DPPH assay results revealed the effectiveness of ethyl acetate fraction on free radical scavenging which was closely related to the reference agent (ascorbic acid), followed by methylene chloride, butanol, crude methanol extract, hexane and the least effective aqueous fractions. While according to SOD-like activity results (Figure 3), butanol fraction was the most potent with inhibition percent (77.3%) compared with the reference agent (ascorbic acid) (75.1%) then the ethyl acetate (ea), aqueous, methylene chloride(mc), crude methanol extract and at last n-hexane fractions. This *in vitro* powerful antioxidant activity of *A. graecorum* was attributed to the presence of the effective alkaloids and the antioxidant flavonoid glycosides as *A. graecorum* aerial parts hold isorhamnetin 3-*O*-glucosyl neohesperidoside besides tamarixetin 3-*O*-dirhamnoside [49].

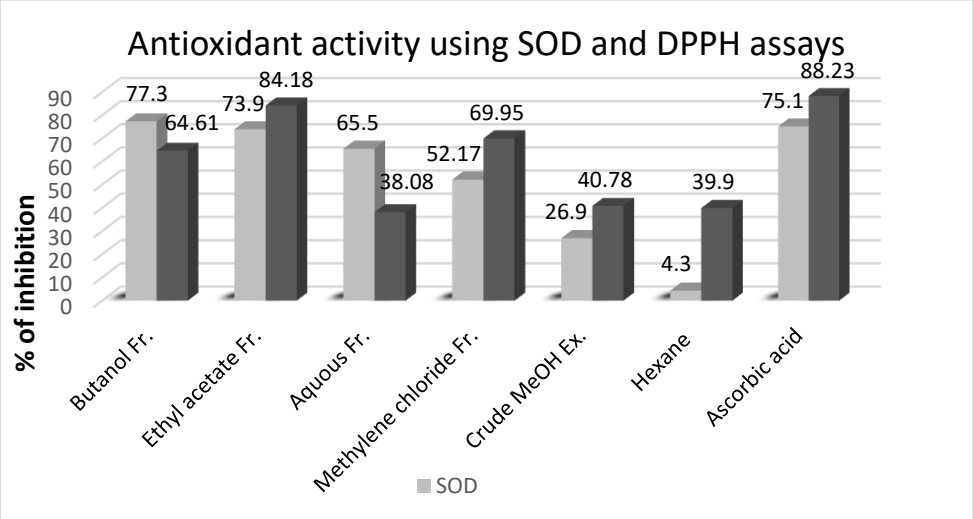

Figure 3: The antioxidant activity of *A. graecorum* extracts

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