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Comparative Phytochemical and Biological Study for *Mesembryanthemum Nodiflorum* and *Aptenia Cordifolia* Plants Growing in Egypt

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IN THIS work, Evaluation of the biological activity and the chemical composition of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* growing in Egypt were carried out. The chemical composition of both plants was evaluated by GC/MS and HPLC/MS analysis. GC/MS analysis of the unsaponifiable matter of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* resulted in the identification of 17 and 13 compounds; respectively, the major compounds were octadecane and neophytadiene; respectively. GC/MS analysis of the saponifiable matter of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* resulted in the identification of 12 and 18 compounds; respectively. The palmitic acid was the most abundant saturated fatty acid in both species. HPLC/MS analysis of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* resulted in the identification of 36 and 48 compounds; respectively. Finally biological studies indicated that the extract of *Mesembryanthemum nodiflorum* has more potent analgesic effect and also showed significant inhibitory activity against colon, cervix, liver and normal melanocyte carcinoma. The 70% ethanol extracts of the aerial parts of plants under investigation have a significant hypoglycemic effect, anti-oxidant, anti-inflammatory and hepatoprotective activity; where *Aptenia cordifolia* extract was more potent than *Mesembryanthemum nodiflorum* extract.

Keywords: *Mesembryanthemum nodiflorum*; *Aptenia cordifolia*; HPLC/MS; GC/MS; Biological studies

Introduction

Plants have been used as a source of traditional medicine for treat of various diseases for many years. They have been considered as excellent source of phytochemicals which showed antioxidant and anticancer activities [1]. The Aizoaceae is the largest succulent plant family in the world [2]. The Family Aizoaceae (Fig-marigold family or Ice plant family) comprises 143 genera and about 2300 species [3] distributed mostly in the arid and semi-arid regions of Africa and few of them are distributed in Asia, Australia and the central Pacific regions. Majority of the species (96%) are endemic to southern Africa [4].

Literatures have shown that many species of family Aizoaceae contain sterols and Flavonoids [5]. An antifungal tetraterpenoid named Trianthenol 1 has been isolated from the

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chloroform extract of *Trianthema portulacastrum* [6]. Six mesembrine alkaloids were identified as constituents of *Sceletium subvelutinum* [7]. Anti-cancer compounds were isolated from the Mesembryanthemum genus, in particular *Mesembryanthemum tortuosum* (Sceletium tortuosum) [8].

This study includes two plants: *Mesembryanthemum nodiflorum* and *Aptenia cordifolia*. Mesembryanthemum is a genus of about 100 species distributed along the coasts of western North America, southern Europe, Africa and the Middle East. Several species are grown as ornamental plants [9]. Mesembryanthemum species are halophytes widely found in semi-arid zones in the northern part of Egypt, Saudi Arabia and Kuwait. They are used as food and used in traditional medicine for treatment of liver diseases, diabetes and ocular infections [10]. Literature review of *Mesembryanthemum nodiflorum*, reported its activity as antiviral and antimicrobial plant but there were no reports about its chemistry [11]. Aptenia is small genus in family Aizoaceae. It is native to South Africa and naturalized in Australia, it contains 4 species cultivated as an ornamental plant in different regions of the world. Literature review of *Aptenia cordifolia,* reported that the leaf extract contains sterols, oxyneolignans, lignans and amides [3]. But no reports were found about its biological activity.

Thus, the objective of this work was tentative identification of secondary metabolites in *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* using HPLC /MS and GC/MS and evaluation of their biological activities

Experimental

Plant Material

Mesembryanthemum nodiflorum was obtained from El Alamein, a town in the northern Matrouh Governorate of Egypt. *Aptenia cordifolia* was obtained from a farm, Beni-Suef, Egypt. Botanical identification of both plants were authenticated by Prof. Dr. Abdelhalim Mohammed; Flora department, Agricultural museum, Cairo, Egypt. Voucher specimens were kept in the Agricultural museum.

General Experimental Procedures

The GC-MS analysis was carried out using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center.

The HPLC-ESI-MS analysis was carried out using a Thermo instrument MS system coupled to a Thermo instruments HPLC system stands at Environmental Studies and Research Institute at Sadat City.

The biological studies include toxicological study and investigation of antihyperglycemic, antioxidant, analgesic, anti-inflammatory, hepatoprotective activities of the ethanolic extracts of aerial parts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* were carried out at National Research Center and investigation of cytotoxic activity was carried out at National Cancer Institute.

Extraction

For GC/MS:

About 100 g of the air-dried powdered of the 2 species were exhaustively extracted with n-hexane. The solvent was evaporated at 40°C under reduced pressure to yield 10 g lipoidal matter.

For HPLC/MS:

Ethanolic extracts of the aerial parts of both species were prepared by macerating 400 gm of the powdered parts separately in successive portions of 70% ethanol till exhaustion. The ethanolic extract in each sample was filtered and evaporated on a Rotary evaporator under reduced pressure to obtain a semisolid residue.

For Biological Study:

400 g of the air –dried powdered aerial parts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* were extracted by cold maceration with 70% ethanol. The solvent, in each case, was evaporated to dryness, under reduced pressure. The percentage yields of solvent-free extracts were 5 and 4g /100g calculated on dry weight for *Mesembryanthemum nodiflorum* and *Aptenia cordifolia*, respectively. The dried extracts were kept in tightly sealed sample tubes for the biological study.

GC-Mass Spectral Analysis

Preparation of fatty acid methyl esters and GC/MS analysis of the prepared fatty acid methyl esters were done according to previous literature (12).

HPLC/ MS

Chemicals:

All solvents and reagents from various suppliers were of the highest purity needed for each application. Methanol and water were of HPLC grade (J.T. Baker, Phillisperg, NJ, USA). Absolute methanol and Formic acid were purchased from Merck (Darmstadt, Germany)

Sample Preparation:

100 µL of extract of each sample dissolved in 1 ml of MeOH. Samples filtrated by TEFLON 0.22 membrane filters. A clean supernatant was evaporated to dryness with nitrogen and reconstituted with 1 mL of mobile phase A (Pure HPLC water+0.1% formic acid), filtered through a PTFE 0.2 µm filter and injected into the HPLC-MS/MS/MS system under the conditions reported.

HPLC-Mass Spectral Analysis:

The HPLC-MS system consisted of electrospray ionization (ESI) interfaced Bruker Daltonik Esquire-LC Amazon SL Ion Trap Mass spectrometer (Bremen, Germany) and Dionex Ultimate 300 (Germany), composed of a quaternary pump with an online degasser, a thermostated column compartment, a photodiode array detector (DAD), an auto sampler, and hystar software .Separation was carried by a dionex bounded silica C8 column (4.6× 150 mm 1.8 um) at a flow rate of 0.4 ml/min. The column oven temperature was set at 30°c.Nitrogen was used as nebulizing gas at a pressure of 45 PSI and the flow rate was adjusted to 10 l/min. The heated capillary was maintained at 350°c. Mobile phase consisted of two solvents, (A) Pure HPLC Water+0.1%Formic acid and (B) AcetonitrilL + 0.1%Formic acid.

Biological Studies

Experimental Animals:

Albino mice of 25-30 g body weight and Adult male albino rats of Sprauge Dawly Strain of 130-150 g body weight were kept under the same hygienic conditions and on a standard laboratory diet consisting of vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%), cellulose (0.2%), casein-95% pure (10.5%) and starch (54.3%).

Standards for Biological Studies:

Indomethacin was obtained from Epico, Egyptian Int. Pharmaceutical industries Co.,A.R.E.,underlisence of MERK &Co. INC-RAHAWY,N.J. USA., Carrageenan and Alloxan were obtained from Sigma Co. Metformin (Cidophage) ® was obtained from Chemical Industries Development (CID), Giza, ARE. It is available in the form of tablets; each contains 500 or 850 mg metformin hydrochloride. Vitamin E (dl α-tocopheryl acetate) was obtained from Pharco Pharmaceutical Co. It is available in the form of gelatinous capsules; each contains 400 mg vitamin E. Carbon tetrachloride (analar) was used.

Biochemical Kits and Reagents:

Biomerieux kit: used for the assessment of blood glucose level. Glutathione kit: used for the assessment of antioxidant activity. Transaminase kit (Biomerieux Co.): used for the assessment of serum AST(SGOT),ALT(SGPT) and ALP

Human Tumor Cell Lines:

HEPG2 (Human liver carcinoma cell line). HFB4 (Normal melanocyte cell line). HELA (Cervix carcinoma cell line). MCF7 (Breast carcinoma cell line). HCT116 (Colon carcinoma cell line). HEP2 (Larynx carcinoma cell line).

Statistical analysis

All analyses were carried out in triplicate and the experimental data were expressed as means ±SD. The software SPSS V14 was used to compare the results by the analysis of variance with one factor (ANOVA). A level of $p < 0.01$ was considered to be significant.

Results and Discussion

GC-MS analysis of the identified unsaponifiable matter of Mesembryanthemum nodiflorum

GC/MS analysis of the unsaponifiable matter of *Mesembryanthemum nodiflorum* revealed the identification of 17 compounds constituting 94.24% of the total composition of the unsaponifiable fraction of the plant extract. The major compound representing (16.93%) is Octadecane.Total identified hydrocarbons were 7 compounds, representing 58.35% of the total identified unsaponifiable compounds of the plant extract, mainly attributed to Octadecane (16.93%). Total identified oxygenated hydrocarbon were 4 compounds, representing 20.43 % of the total identified unsaponifiable compounds of the plant extract, mainly attributed to 2-ethyl Hexanol (14.29%).Total identified terpenes were 5 compounds, representing 13.78% of the total identified unsaponifiable compounds of the plant extract, mainly attributed to Betulin (5.38%). The detected phytosterol was Stigmasterol which represented 1.68% of the total identified unsaponifiable compounds of the plant extract. Table 1 represents the results.

GC-MS analysis of the identified unsaponifiable matter of Aptenia cordifolia

GC/MS analysis of the unsaponifiable matter of *Aptenia cordifolia* revealed the identification of 13 compounds constituting 99.3% of the total composition of the unsaponifiable fraction of the plant extract. The major compounds representing (27%) is Neophytadiene. Total identified

TABLE 1. Results of GC-MS analysis of the identified unsaponifiable matter of Mesembryanthemum nodiflorum

No.	Rt.	RRt.	Area %	Compounds	Molecular formula
1	6.94	0.18	14.29	2-ethyl hexanol	$C_8H_{18}O$
2	27.93	0.74	1.34	Pentadecanal	$C_{15}H_{30}O$
3	28.11	0.75	2.58	Tetradecane	$C_{14}H_{30}$
4	30.85	0.82	2.69	Neophytadiene	$C_{20}H_{38}$
5	31.12	0.83	3.24	6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$
6	31.94	0.85	1.46	Phytol	$C_{20}H_{40}$
7	33.29	0.88	11.39	Hexadecane	$C_{16}H_{34}$
8	34.80	0.93	1.59	1-Docosene	$C_{22}H_{44}$
9	34.91	0.93	2.10	Heptadecane	$C_{17}H_{36}$
10	37.26	0.99	13.64	9-Octadecene	$C_{18}H_{38}$
11	37.41	1	16.93	Octadecane	$C_{18}H_{36}$
12	38.27	1.02	1.56	2 Hexyl-1-decanol	$C_{16}H_{34}O$
13	42.79	1.14	10.12	4,8,12,16-Tetramethylpentadecan	$C_{19}H_{40}$
14	52.34	1.39	2.58	α-Neooleana-3-(5),12-diene	$C_{30}H_{48}$
15	54.80	1.46	1.67	α-Amyrin	$C_{30}H_{50}O$
16	55.08	1.47	5.38	Betulin	$C_{30}H_{50}O_2$
17	55.88	1.49	1.68	Stigmasterol	$C_{29}H_{48}O$
			Hydrocarbons		58.35
			Oxygenated hydrocarbons		20.43
			Total terpens		13.78
			Total sterols		1.68
			Unidentified compounds		5.76

hydrocarbons were 2 compounds, representing 10% of the total identified unsaponifiable compounds of the plant extract, mainly attributed to Hexadecane (6%). Total identified oxygenated hydrocarbons were 3 compounds, representing 16.7% of the total identified unsaponifiable compounds of the plant extract, mainly attributed to 6, 10, 14-trimethyl-2-Pentadecanone (9.7%).Total identified terpenes were 3 compounds, representing 42% of the total identified unsaponifiable compounds of the plant extract, mainly attributed to Neophytadiene (27%). The identified Phytosterols were 5 compounds, representing 30.6% of the total identified unsaponifiable compounds of the plant extract, mainly attributed to Stigmastan-3, 5-diene (10%). Table 2 represents the results.

GC-MS analysis of the identified saponifiable matter (FAME) of Mesembryanthemum nodiflorum

The GC-MS analysis of the saponifiable matter of *Mesembryanthemum nodiflorum* revealed identification of 12 compounds constituting 82.13% of the total composition of the saponifiable fraction. Generally the percentage of the total identified saturated fatty acids (63.6%) is higher than the percentage of total identified unsaturated fatty acids (18.53%). The results showed that the palmitic acid is the most abundant saturated fatty acid. Table 3 represents the results.

Matter (FAME) of Aptenia cordifolia

The GC-MS analysis of the saponifiable matter of *Aptenia cordifolia* revealed identification of 18 compounds constituting 98.5% of the total composition of the saponifiable fraction. Generally the percentage of the total identified saturated fatty acids (76.25%) is higher than the percentage of total identified unsaturated fatty acids(22.33%).The results showed that the the palmitic acid is the most abundant saturated fatty acid. Table 4 represents the results.

TABLE 2. Results of GC-MS analysis of the identified unsaponifiable matter of Aptenia cordifolia

NO.	Rt.	RRt.	Area %	Compounds	Molecular formula
1	16.32	0.52	2.9	1,1-diethoxy pentane	$C_9H_{20}O_2$
2	30.86	1	27	Neophytadiene	$C_{20}H_{38}$
3	31.12	1.008	9.7	6,10,14-Trimethyl-2-pentadecanone	$C_{18}H_{36}O$
4	31.49	1.02	4.1	3,7,11,15Tetramethyl2hexadecen-1-ol	$C_{20}H_{40}O$
5	31.95	1.03	10.7	Phytol	$C_{20}H_{40}O$
6	34.91	1.13	6	Hexadecane	$C_{16}H_{34}$
7	38.88	1.25	4	9-Octadecene	$C_{18}H_{36}$
8	52.24	1.69	2.9	Campsterol	$C_{28}H_{48}O$
9	53.69	1.73	10	Sitosterol	$C_{29}H_{50}O$
10	54.47	1.76	3.5	Stigmastenol	$C_{29}H_{50}O$
11	55.56	1.8	4.3	Cyclolanostanol	$C_{30}H_{52}O$
12	55.89	1.81	9.4	Cholest-4-ene-3,6-dione	$C_{27}H_{42}O_2$
13	56.82	1.84	4.8	4,14-Dimethyl-9,19-cycloergost-24-en-3-ol	$C_{30}H_{50}O$
				Total hydrocarbons	10
				Oxygenated hydrocarbons	16.7
				Total terpens	42
				Total sterols	30.6
				Unidentified compounds	.07%

TABLE 3. GC-MS analysis of the saponifiable matter; FAMEs of *Mesembryanthemum nodiflorum*

Peak No.	Identified compound	Molecular formula	R_t	RR_t	Area
1	Dodecanoic acid = lauric acid	$C_{12}H_{24}O_2$	17.5	0.61	1.79
2	Tetradecanoic acidr= myristic acid	$C_{14}H_{28}O_2$	23.1	0.81	7.27
3	Pentadecanoic acid	$C_{15}H_{30}O_2$	25.7	0.90	0.57
4	Hexadecanoic acid= palmitic acid	$C_{16}H_{32}O_2$	28.3	1	47.48
5	Heptadecanoic acid=margaric acid	$C_{17}H_{34}O_2$	30.7	1.08	0.46
6	(9*Z*,12*Z*)-9,12-Octadecadienoic acid= Linoleic acid	$C_{18}H_{32}O_2$	32.2	1.13	8.53
7	11Octadecenoic acid=Vaccenic acid	$C_{18}H_{34}O_2$	32.4	1.14	7.47
8	Stearic acid	$C_{18}H_{36}O_2$	33.0	1.16	4.89
9	8,11Octadecadienoic acid	$C_{18}H_{32}O_2$	33.4	1.18	1.53
10	10Undecenoic acid, 2methoxy	$C_{11}H_{20}O_2$	35.3	1.24	1.00
11	Hexanoic acid=caproic acid	$C_6H_{12}O_2$	37.7	1.33	0.43
12	4,8,12,16-Tetramethylheptadecanoic acid	$C_{21}H_{42}O_2$	38.0	1.34	0.87
	Saturated fatty acid				63.6%
	Unsaturated fatty acid				18.53%
	Un identified compounds				17.87%

TABLE 4. GC-MS analysis of the saponifiable matter; FAMEs of Aptenia cordifolia

PeakNo.	Identified compound	Molecular formula	R_t	RR_t	Area
1	Tetradecanoic acid= *Myristic acid*	$C_{14}H_{48}O_2$	23.2	0.81	0.73
2	Pentadecanoic acid	$C_{15}H_{30}O_2$	25.8	0.91	0.57
3	5,9,13-Trimethyl-tetradecanoic acid	$C_{17}H_{34}O_2$	26.7	0.94	0.12
4	Hexadecanoic acid=Palmitic acid	$C_{16}H_{32}O_2$	28.3	1	63.99
5	Hexadecanoic acid, 15methyl	$C_{17}H_{34}O_2$	30.0	1.06	0.09
6	Heptadecanoic acid= margaric acid	$C_{17}H_{34}O_2$	30.7	1.08	0.92
7	(9*Z*,12*Z*)-9,12-Octadecadienoic acid= Linoleic acid	$C_{18}H_{32}O_2$	32.2	1.13	9.11
8	9,12,15Octadecatrienoic acid=α-Linolenic acid	$C_{18}H_{30}O_2$	32.4	1.14	12.82
9	Octadecanoic acid=stearic acid	$C_{18}H_{36}O_2$	33.0	1.16	6.77
10	2Ethylbutyric acid	$C_6H_{12}O_2$	33.9	1.19	0.50
11	6,9Octadecadienoic acid	$C_{18}H_{32}O_2$	34.5	1.21	0.42
12	Hexanoic acid, octadecyl ester	$C_{24}H_{48}O_2$	34.7	1.22	0.25
13	Oxiraneundecanoic acid, 3pentyl, methyl ester	$C_{19}H_{36}O_3$	35.3	1.24	0.38
14	Eicosanoic acid=Arachidic acid	$C_{20}H_{40}O_2$	37.4	1.32	0.65
15	Methyl4methoxy4,8,12,16tetramethylheptad ecanoate	$C_{23}H_{46}O_3$	38.0	1.34	0.19
16	Docosanoic acid= *Behenic acid*	$C_{22}H_{44}O_2$	41.4	1.46	0.63
17	Tricosanoic acid	$C_{23}H_{46}O_2$	43.3	1.53	0.09
18	Tetracosanic acid=lignoceric acid	$C_{24}H_{48}O_2$	45.14	1.59	0.46
	Saturated fatty acid				76.25
	Unsaturated fatty acid				22.33
	Un identified compounds				1.5

HPLC/DAD/ESI-MS for Mesembryanthemum nodiflorum

Table 5 shows thirty six compounds identified from *Mesembryanthemum nodiflorum*. Nine terpens, eight phenolic acids and other phenolic compounds, seven flavonoids, five sterols, three lignans, three alkaloids and one hydrocarbon compound.

HPLC/DAD/ESI-MS for Aptenia cordifolia

Table 6 shows forty eight compounds were identified from *Aptenia cordifolia*. Sixteen flavonoids, fifteen terpens, five phenolic acid and other phenols, two alkaloids, three sterols, three polyprenols, one glucosinolate compound and one coumarin compound.

Biological studies

Toxocolgical studies

Determination of the LD50 of the aqueous-ethanol extracts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* according to [13]. The LD50 of *Mesembryanthemum nodiflorum* extract was 5.6 g/kg b.wt. While the LD50 of *Aptenia cordifolia* extract was 8.4 g/kg b.wt.

Antihyperglycemic activity

Hyperglycemia is considered to be the dominant pathological characteristic of Diabetes mellitus (DM), as it causes the majority of the symptoms associated with DM. Chronic hyperglycemia results in the accumulation of advanced glycation end products)AGEs) in the body, which further increase the risk for vascular complications in patients with DM as damaged kidney tissues, including reduced glomeruli, dilation of the renal capsule and kidney tubules[14]. Investigation of the antidiabetic activity of the 70% ethanol extracts of the aerial parts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* [15, 16]. Samples for investigation of antihyperglycemic activity were taken after 2 weeks and 4 weeks. The 70% ethanol extracts of the aerial parts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* showed a valuable hypoglycemic effect

TABLE 5.Metabolites putatively identified by HPLC/MS analysis in *Mesembryanthemum nodiflorum*

Peak No.	R_t	Mass	ESI-HRMS (m/z)	Molecular formula	Tentative identification	
	0.7	922	923.4	$C_{44}H_{74}O_{20}$	Capsianoside VI	M+H
	1.4	750	751.5	$C_{46}H_{70}O_8$	1,26-Hexacosanediol diferulate	M+H
	3.5	650	651.4	$C_{38}H_{58}O_{10}$	Arjunolic acid 3-glucoside	M+H
	10.1	704	705.2	$C_{36}H_{32}O_{15}$	Occidentoside	M+H
	10.4	660	661.5	$C_{46}H_{76}O_2$	Campesteryl α-linolenate	M+H
	10.8	814	813.6	$C_{51}H_{90}O_7$	Sitoindoside I	M-H
	11.1	917	918.1	$C_{64}H_{116}O_2$	α-Amyrin tetratriacontanoate	M+H
	15.1	702	701.3	$C_{34}H_{38}O_{16}$	Ramontoside	M-H
	15.2	610	609.7	$C_{27}H_{30}O_{16}$	Rutin	M-H
	15.9	361	362.3	$C_{24}H_{43}NO$	2,4,14-Eicosatrienoic acid isobutylamide	M+H
	18.0	814	813.3	$C_{35}H_{42}O_{22}$	Kaempferol 3-[2''-glucosyl-6''-acetyl-galactoside] 7-glucoside	M-H
	18.1	904	903.6	$C_{45}H_{76}O_{18}$	Torvoside A	M-H
	18.3	722	721.4	$C_{39}H_{62}O_{12}$	Ophiopogonin C	M-H
	18.5	114	114.2	C_8H_{18}	Octane	M
	18.7	248	249.1	$C_{14}H_{16}O_4$	3,4-Dihydroxy-5-prenylcinnamic acid	M+H
	18.9	764	765.3	$C_{40}H_{60}O_{14}$	Spinacoside D	M+H
	19.3	652	653.7	$C_{45}H_{80}O_2$	β-Sitosterol palmitate	M+H
	21.3	726	727.4	$C_{34}H_{34}N_2O_{16}$	Lampranthin II	M+H
	21.5	165	164.9	$C_{10}H_{15}NO$	Hordenine	M-H
	21.5	222	223.2	$C_{15}H_{10}O_2$	2-Phenyl-4-benzopyron	M+H
	21.8	208	209.0	$C_{11}H_{12}O_4$	Methyl ferulate	M+H
	22.4	226	227.1	$C_{13}H_{22}O_3$	(3R,9R)-3,9-dihydroxymegastigm-5-*en*-4-one	M+H
	22.5	314	313.7	$C_{18}H_{18}O_5$	Apteniol A	M-H
	23.1	276	277.0	$C_{10}H_{12}O_7S$	Dihydroferulic acid 4-sulfate	M+H
	23.6	754	753.7	$C_{34}H_{42}O_{19}$	1,2-Disinapoylgentiobiose	M-H
	24.2	488	487.9	$C_{30}H_{48}O_5$	Tormentic acid	M-H
	24.5	181	180..8	$C_9H_{10}O_4$	Dihydrocaffeic Acid	M-H
	24.7	594	595.4	$C_{27}H_{30}O_{15}$	Kaempferol 3-*O*-rutinoside	M+H
	24.8	432	431.0	$C_{21}H_{20}O_{10}$	Apigenin-7-*O*-glucoside	M-H
	25.6	436	435.6	$C_{20}H_{20}O_{11}$	Taxifolin-3-O-α-L-arabinofuranoside	M-H
	25.8	268	267.2	$C_{18}H_{36}O$	6, 10, 14-Trimethylpentadecan-2-one	M-H
	26.8	898	897.6	C47H78O16	Meliltoside	M-H
	27.6	136	136.8	$C_{10}H_{16}$	Isoterpinolene	M
	27.8	362	363.1	$C_{19}H_{22}O_7$	2', 8-Dihydroxy-3', 4', 5', 7-tetramethoxyflavan	M+H
	28.0	166	167.9	$C_9H_{10}O$	4-Hydroxy-dihydrocinnamic acid	M+H
	28.1	154	155.0	$C_7H_6O_4$	3, 4-Dihydroxybenzoic acid	M+H

with percentage of inhibition after 2 weeks (24.8%, 31.6%; respectively) and after 4 weeks (45.2%, 52.5%; respectively) as well as the reference drug metformin with percentage of inhibition after 2 and 4 weeks (36.3%, 67.2%; respectively). *Aptenia cordifolia* ethanolic extract was more potent than *Mesembryanthemum nodiflorum* ethanolic extract. The results are shown in Fig. 1.

Antioxidant activity

Antioxidants are considered as a group of compounds which are responsible for inhibition lipid and other biomolecules oxidation. As a result of this mechanism they prevent or repair the damage of body cells caused by oxygen. Many studies have focused on natural antioxidants and their usage in food systems to inhibit oxidation [17]. Investigation of the antioxidant activity of the 70% ethanol extracts of the aerial parts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* [15, 16, 18]. Percent of change of blood glutathione level in diabetic group was 41.4% while both ethanolic extracts were 4.3% and 3.2%. Both plants show percentage of change nearly similar to that of vit. E 1.3%. The ethanolic extract of *Aptenia cordifolia* shows more potent response as antioxidant than *Mesembryanthemum nodiflorum*. The results are shown in Fig. 2.

TABLE 6. Metabolites putatively identified by HPLC/MS analysis in *Aptenia cordifolia.*

Peak No.	R_t	Mass	ESI-HRMS (m/z)	Molecular formula	Tentative identification	
	0.2	824	825.5	$C_{42}H_{64}O_{16}$	Oleragenoside	M+H
	0.4	1290	1291.4	$C_{56}H_{90}O33$	Rebaudioside M	M+H
	1.3	726	727.5	$C_{34}H_{38}N_2O_{16}$	Lampranthin II	M+H
	1.4	678	679.3	$C_{36}H_{54}O_{12}$	Medicagenic acid 3-O-b-D-glucuronide	M+H
	2.2	698	699.7	$C_{50}H_{82}O$	Arachisprenol 10	M+H
	7.6	248	249.1	$C_{14}H_{16}O_4$	7-hydroxy-6-(3-hydroxy-3-methylbutyl)-2H-chromen-2-one	M+H
	11.6	844	845.4	$C_{38}H_{52}O_{21}$	(+)-Pinoresinol 4-O-[beta-D-Glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside]	M+H
	11.8	448	449.4	$C_{21}H_{20}O_{11}$	kaempferol 7-O-glucoside	M+H
	13.5	680	679.9	$C_{36}H_{56}O12$	Suavissimoside R1	M-H
	13.8	822	821.4	$C_{39}H_{66}O_{18}$	Loquatifolin A	M-H
	13.8	664	665.1	$C_{37}H_{28}O_{12}$	ent-Epiafzelechin(2a→7,4a→8)epiafzelechin 3-(4-hydroxybenzoic acid)	M+H
	13.9	834	833.6	$C_{57}H_{86}O_4$	3-Decaprenyl-4,5-dihydroxybenzoate	M-H
	14.5	872	873.4	$C_{44}H_{72}O_{17}$	Capsicoside C2	M+H
	14.8	642	641.4	$C_{32}H_{50}O_{13}$	Steviobioside	M-H
	16.3	824	825.1	$C_{43}H_{36}O_{17}$	Kaempferol 3-(3’’,6’’-diacetyl-2’’,4’’-di-p-coumaroylrhamnoside)	M+H
	16.4	948	949.6	$C_{47}H_{80}O_{19}$	Notoginsenoside H	M+H
	16.6	838	837.2	$C_{39}H_{50}O_{20}$	3,5,7-Trihydroxy-4’-methoxy-8-prenylflavone 3-[rhamnosyl-(1→6)-galactoside] 7-galactoside	M-H
	16.7	800.3	801.3	$C_{38}H_{40}O_{19}$	Isoscoparin 2’’-(6-(E)-ferulylglucoside)	M+H
	18.6	912	913.4	$C_{46}H_{72}O_{18}$	Medicagenic acid 28-O-[b-D-xylosyl-(1→4)-a-L-rhamnosyl-(1→2)-a-L-arabinosyl] ester	M+H
	19.5	902	903.5	$C_{45}H_{74}O_{18}$	Tuberoside E	M+H
	19.8	165	164.8	$C_{10}H_{15}N$	Hordenine	M-H
	20.3	704	703.7	$C_{50}H_{88}O$	Glycinoprenol 10	M-H
	20.9	848	849.7	$C_{58}H_{88}O_4$	3-Decaprenyl-4-hydroxy-5-methoxybenzoate	M+H
	21.1	208	209.0	$C_{11}H_{12}O_4$	Methyl ferulate	M+H
	21.4	769	768.2	$C_{38}H_{41}O_{17}$	Peonidin 3-(6’’-p-coumaroyl-glucoside) 5-glucoside	M-H
	21.8	662	661.3	$C_{36}H_{38}O_{12}$	5’,5’’’,8,8’’-Tetrahydroxy-3’,3’’’,4’,4’’’,7’,7’’-hexamethoxy-5,5’’-biflavan	M-H
	22.0	936	937.1	$C_{43}H_{36}O_{24}$	Theaflavonin	M+H
	22.4	702	701.3	C34H38O16	Ramontoside	M-H
	22.4	1100	1101.6	$C_{50}H_{84}O_{26}$	Capsianoside III	M+H
	22.4	766	765.7	$C_{55}H_{90}O$	Arachisprenol 11	M-H
	22.6	579	580.2	$C_{27}H_{31}O_{14}$	Pelargonidin 3-rhamnoside-5-glucoside	M+H
	23.1	438	437.1	$C_{26}H_{46}O_5$	27-Nor-5b-cholestane-3a,7a,12a,24,25-pentol	M-H
	23.1	150	150.0	$C_9H_{10}O_2$	Dihydrocinnamic acid	M
	23.4	740	739.1	$C_{39}H_{32}O_{15}$	Kaempferol 3-(2”,6”-di-(E)-p-coumarylglucoside)	M-H
	24.7	972	971.4	$C_{47}H_{72}O_{21}$	Betavulgaroside VI	M-H
	24.9	594	593.1	$C_{27}H_{30}O_{15}$	Kaempferol-3-O-rutinoside	M-H
	25.0	898	899.6	$C_{24}H_{78}O_{16}$	Chondrillasterol 3-[glucosyl-(1→2)-glucosyl-(1→2)-glucoside]	M+H
	25.4	460	459.3	$C_{28}H_{44}O_5$	3,5,9,14-Tetrahydroxyergosta-7,22-dien-6-one	M-H
	25.8	933	934.3	$C_{43}H_{49}O_{23}$	Petanin	M+H
	26.0	124	124.1	C7H8O2	4-(Hydroxymethy)phenol	M
	26.1	664	665.3	$C_{36}H_{56}O_{11}$	Elatoside G	M+H
	27.0	940	939.4	$C_{46}H_{68}O_{20}$	Betavulgaroside VIII	M-H
	27.3	740	739.0	$C_{33}H_{40}O_{19}$	Kaempferol-3-glucoside-2’’rhamnoside-7-rhamnoside	M-H
	27.4	676	677.2	$C_{37}H_{40}O_{12}$	5’,8,8’’-Trihydroxy-3’,3’’’,4’,4’’’,5’’’,7’,7’’-heptamethoxy-5,5’’-biflavan	M+H
	27.9	288	287.0	$C_{15}H_{12}O_6$	2,4’,5,7-Tetrahydroxyflavanone	M-H
	28.5	373	374.1	$C_{11}H_{18}NO_9S_2$	Gluconapin	M+H
	29.5	742	741.1	$C_{32}H_{38}O_{20}$	Quercetin 3-(2Gal-apiosylrobinobioside)	M-H
	29.6	636	635.4	$C_{35}H_{56}O_{10}$	Madlongiside C	M-H

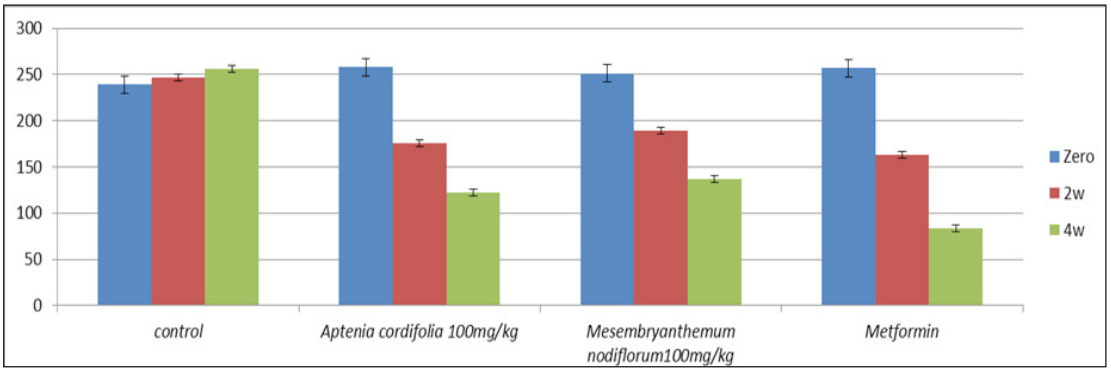

Fig.1. Histogram representing the effect of the 70% ethanolic extracts of the aerial parts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* on blood glucose level in diabetic male albino.

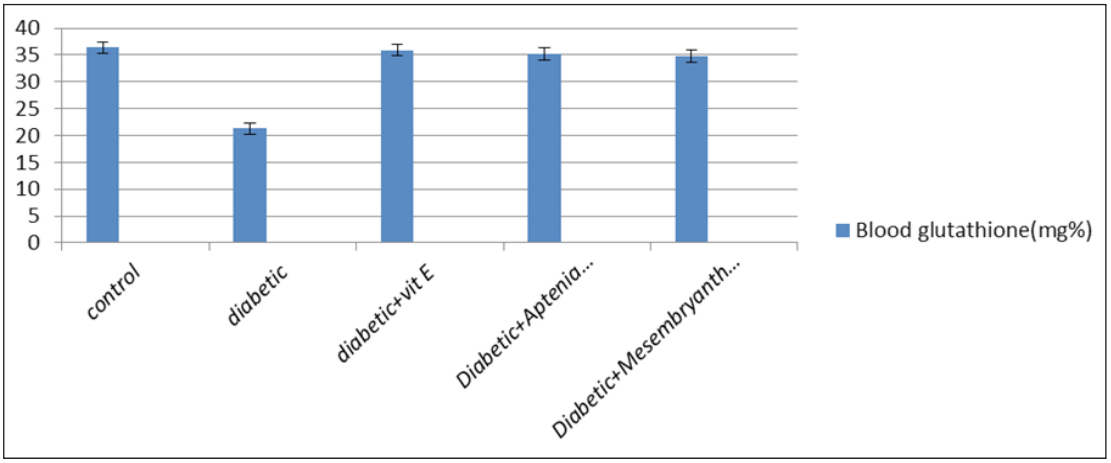

Fig. 2. Histogram representing the antioxidant activity of the 70% ethanol extracts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia*

Analgesic activity

Non-steroidal anti-inflammatory drugs (NSADs) have been used for many years as analgesic and ani-inflammatory. Recent researches cleared that repeated use of NSADs had many undesirable effects as gastrointestinal lesions or renal and liver failure. Therefore, finding new effective and safe analgesic and anti-inflammatory agents become an important need [19]. Investigation of the analgesic activity of the 70% ethanol extracts of the aerial parts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* [20]. Ethanolic extract of *Mesembryanthemum nodiflorum* shows inhibition by 43% which is more potent than ethanolic extract of *Aptenia cordifolia* that shows inhibition by 31.3%, compared with the reference drug Indomethacin® that gives inhibition percent 58.2%. The results are shown in Fig. 3.

Anti-inflammatory activity

Inflammation is known as a symptom of many diseases as arthritis. Arthritis is an inflammatory disease affects the joints and connective tissues like bones, muscles, cartilage and tendons. It has been considered as major medical problem throughout the world [21]. Investigation of the anti-inflammatory activity of the 70% ethanol extracts of the aerial parts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* [22]. Percent of change of both ethanolic extracts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* after 4 hours were 76% and 79 %; respectively. These percentages of inhibition were similar to the effect of Indomethacin 81.9%. The percent of change of *Aptenia cordifolia* was more potent than *Mesembryanthemum nodiflorum*. The results are shown in Fig. 4.

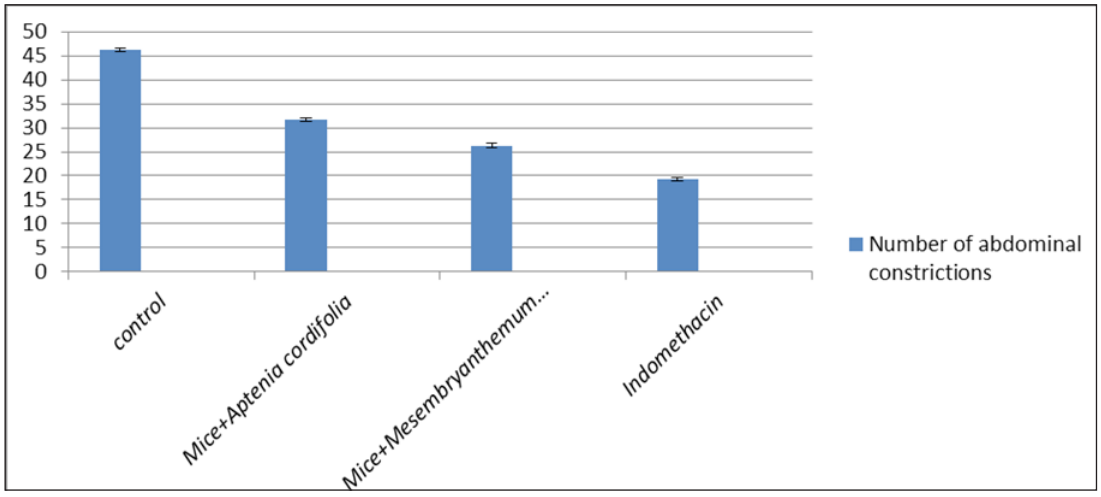

Fig. 3. Histogram representing the analgesic activity of the 70% ethanol extracts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia*

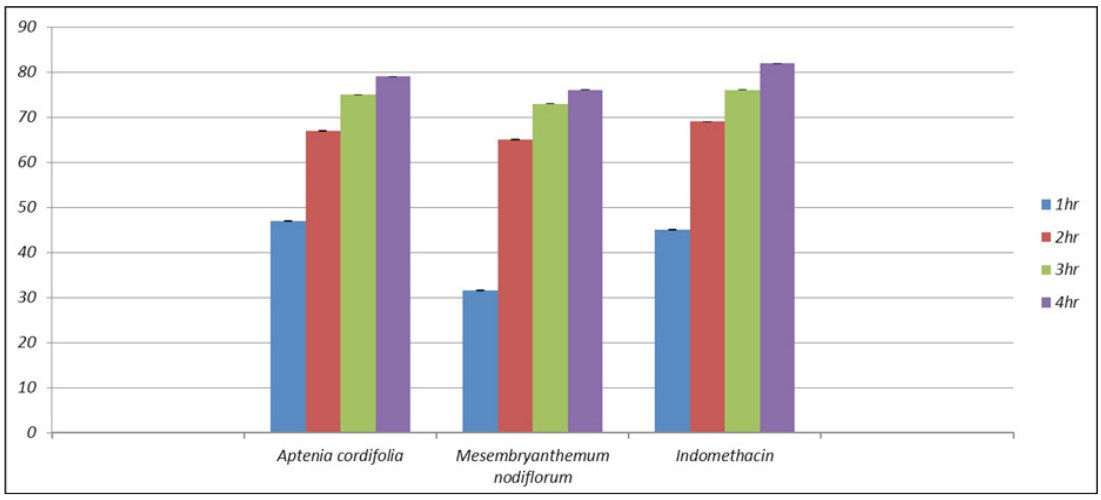

Fig. 4. Histogram representing the anti-inflammatory activity of the 70%ethanol extracts of Mesembryanthemum nodiflorum and Aptenia cordifolia

Hepatoprotective activity

Treatment of liver diseases using synthetic drugs losses their interest because of their serious side effects and insufficiency. While using natural plants in treatment of liver diseases have been proved for their effectiveness and safety [23]. Investigation of the hepatoprotective activity of the 70% ethanol extracts of the aerial parts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* [24, 25]. The results revealed that the given dose of CCl_4 produces significant elevation in AST, ALT and ALP enzymes. The hepatoprotective effects of the extracts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* were compared with those of silymarin. In this view, the reduction in levels of AST, ALP and ALT enzymes by the two extracts is an indication of their effectiveness in repair of hepatic tissue damage. *Aptenia cordifolia* has more hepatoprotective effect more than *Mesembryanthemum nodiflorum*. The results are shown in Fig. 5.

Cytotoxic activity

Cancer is one of the leading causes of deaths worldwide. Treatment of cancer is formed of three main approaches; one of them is chemotherapy along with surgical treatment and radiation therapy. The synthetic chemotherapeutic drugs

are non-specifically distributed in all the body tissues affecting both normal and cancerous cells. Using synthetic chemotherapeutic drugs lead to severe side effects as well as inadequate drug concentration in the tumor cells. Therefore, there is a need to replace synthetic chemotherapeutics with natural products that exhibit the properties to inhibit cancer growth with fewer side effects [26]. Potential cytotoxic activity was measured using the reported method of Skehan, et al., 1990 [27]. *Mesembryanthemum nodiflorum* extract showed significant inhibitory activity against colon, cervix, liver and normal melanocyte carcinoma; while showed low inhibitory activity against larynx carcinoma. *Aptenia cordifolia* showed low inhibitory activity against colon, cervix, liver, larynx and normal melanocyte carcinoma. Both extracts showed negative response against breast carcinoma. Table 7 represents the results.

Conclusion

The GC/MS analysis of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* plants growing

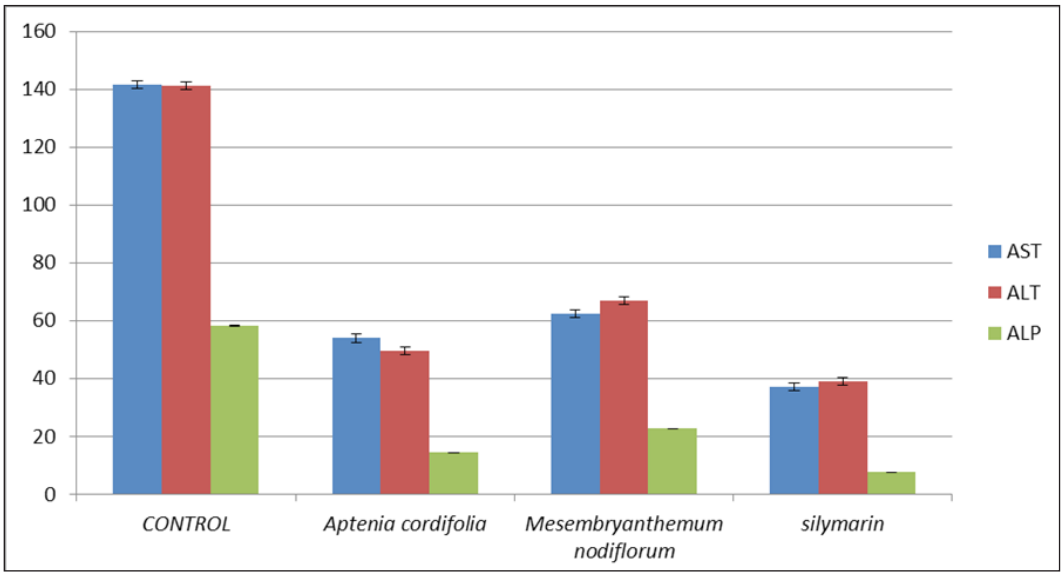

Fig. 5. Histogram representing the hepatoprotective activity of the 70% ethanol extracts of Mesembryanthemum nodiflorum and Aptenia cordifolia

TABLE 7. Results of cytotoxic activity of the alcoholic extract of the aerial parts of *Mesembryanthemum nodiflorum* and *Aptenia cordifolia* on human cancer cell lines

Name of cell line	IC_{50} of *Mesembryanthemum nodiflorum*	IC_{50} of *Aptenia cordifolia*
HEPG2 (Human liver carcinoma cell line)	1.39µ/well	2µg/well
HFB4 (Normal melanocyte cell line	1.01 µg/well	4.86 µg/well
HELA (Cervix carcinoma cell line)	0.705 µg/well	3.11 µg/well
MCF 7(Breast carcinoma cell line)	Negative	
HCT116 (Colon carcinoma cell line)	0.82 µg/well	1.58 µg/well
HEP2 (Larynx carcinoma cell line)	3.49µg/well	3.87µg/well

in Egypt revealed that the; unsaponifiable fractions constituting 94.24% and 99.3% of the total composition of both plants extracts; respectively and the saponifiable fractions constituting 82.13% % and 98.5% of the total composition of both plants extracts; respectively. In HPLC/MS analysis 36 compounds were identified in *Mesembryanthemum nodiflorum* extract and 48 compounds were identified in *Aptenia cordifolia* extract. The biological study of *Mesembryanthemum nodiflorum* ethanolic extract showed potent analgesic effect, and significant inhibitory activity against colon, cervix, and liver and normal melanocyte carcinoma. While *Aptenia cordifolia* ethanolic extract showed potent hypoglycemic effect, anti-oxidant, anti-inflammatory and hepatoprotective activity.

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دراسة مقارنة نباتية وبيوكيميائية وبيولوجية لنباتات الغاسول *Mesembryanthemum nodiflorum* والصبار الاسرائيلي *Aptenia cordifolia* التي تنمو في مصر

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في هذا العمل، تم تقييم النشاط البيولوجي والتركيب الكيميائي لنباتات الغاسول والصبار الاسرائيلي التي تنمو في مصر. تم تقييم التركيب الكيميائي لكلا النباتين من خلال تحليل تحليل كروماتوجرافيا الغاز ذات الكفاءة العالية GC / MS وتحليل كروماتوجرافيا السائل ذات الكفاءة العالية HPLC / MS. نتج عن تحليل كروماتوجرافيا الغاز ذات الكفاءة العالية للاجزاء الغير قابلة للتصبن لنباتين الغاسول والصبار الاسرائيلي تحديد 17 و 13 مركب ؛ على التوالي ، كانت المركبات الرئيسية هي الأوكتاديكان والنيوفيتاديين ؛ على التوالي. نتج عن تحليل تحليل كروماتوجرافيا الغاز ذات الكفاءة العالية للاجزاء القابله للتصبن تحديد 12 و 18 مركب ؛ على التوالي. كان حمض النخيل أكثر الأحماض الدهنية المشبعة وفرة في كلا النوعين. كشفت تحليل كروماتوجرافيا السائل ذات الكفاءة العالية لنباتين الغاسول والصبار الاسرائيلي تحديد 36 و 48 من المركبات. على التوالي. في النهاية، أشارت الدراسات البيولوجية إلى أن مستخلص الغاسول له تأثير مسكن أكثر فعالية وأظهر أيضًا نشاطًا مثبطًا كبيرًا ضد سرطان القولون وعنق الرحم والكبد وسرطان الخلايا الميلانينية الطبيعي. مستخلصات الإيثانول 70 ٪ من الأجزاء الهوائية من النباتات قيد الدراسه لها نشاط كبير ضد سكر الدم ، ومضادات الأكسدة ، والنشاط المضاد للالتهابات والنشاط الواقي للكبد. حيث كان مستخلص الصبار الاسرائيلي أكثر فعالية من مستخلص الغاسول.