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Qualitative GC-MS analysis and antimicrobial activity of volatiles from *Carthamus lanatus* (L.) growing in Egypt

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Abstract

The volatiles of both fresh aerial parts and flowers of *Carthamus lanatus* L. plant growing in Egypt were isolated by hydro-distillation method. The obtained volatiles were subsequently subjected to qualitative analysis by GC-MS analysis, which in turn revealed the presence of 44 compounds. The interpretation of the isolated compounds depended on comparison of their mass spectra with those saved in instrument library, alongside the data reported in literature. On the other hand, a comparative investigation between the Egyptian and Bulgarian species was carried out, which in turn revealed great differences. Antimicrobial screening (using agar diffusion method) of the screened volatiles of Egyptian plant was also carried out, that labored into promising results of potent activity exhibited against different bacteria and fungi compared with gentamycin and ketoconazole antimicrobial standards.

Keywords: *Carthamus lanatus*, GC-MS, Asteraceae, Antimicrobial activity, agar diffusion method.

1. Introduction

Asteraceae is one of the largest and economically most important plant families. It is divided into eleven subfamilies and thirty-five tribes. Genus *Carthamus* is a well-known genus belongs to Asteraceae that composed of annual or perennial herbs or shrublets, usually spiny with homogamous capitula and compressed very hard achenes (Formisano *et al.*, 2012). *Carthamus lanatus* L. is a biennial plant growing in the Mediterranean region (Hellwig, 2004). Previous studies on this plant revealed its importance due to presence of several different components of diverse chemical nature. Phytochemical studies showed the presence of flavonoids (El-Shaer *et al.*, 1998; Novruzov and Shamsizade, 1998), sesquiterpenes glycosides (San Feliciano *et al.*, 1990), lipids (Demir *et al.*, 1978 ; Stefanov *et al.*,2003), aromatic acids (Lahloub *et al.*,1993), sterols, triterpenes and volatiles (Mitova *et al.*,2003). Recently, alkaloids, tannins and saponins were reported (Feroz and Ali.,2016).

The most important biological activities reported for *Carthamus lanatus* L. include antioxidant (Kancheva *et al.*, 2007), analgesic, anti-inflammatory (Bocheva *et al.*, 2003; Jalil *et al.*, 2003), sedative, antitumor (Benedi *et al.*, 1986), antibacterial and antifungal activities (Taskova *et al*., 2002). Moreover, mitogenic effect (Topashka-Ancheva *et al*., 2006) and clastogenic effect (Topashka-Ancheva. *et al*., 2003) were evaluated.

In 2003, a GC-MS study of the volatiles of the Bulgarian plant showed the presence of many volatile components (Mitova *et al.*, 2003), however, to the best of our knowledge, no studies have been reported in literature concerning analysis of the volatile components of the Egyptian plant. Thus, the study in hand aimed, for the first time, to analyze the volatiles of Egyptian plant, compare its volatiles with that of the Bulgarian one, and to evaluate the antimicrobial activity of volatiles present.

2. Experimental

2.1.Plant material and chemicals

The aerial flowering parts of *Carthamus lanatus* (L.) were collected in June 2016 from Borg Al-Arab region, Alexandria, Egypt. The plant was used in fresh state. The plant was kindly identified by Prof. Dr. Ahmed A. Seif El-Din. Professor of Pharmacognosy, Department of Pharmacognosy, Alexandria University, Egypt. Solvents and chemicals were purchased from Merck, Dramstadt, Germany.

2.2.Instruments

GC-MS analysis was performed with Agilent Technologies 7890A gas chromatography system (Agilent, United States) linked to Agilent Technologies 5975C inert mass spectrometry detector provided with Agilent Mass Hunter™ Acquisition software (Agilent, United States).

2.3.Isolation of volatile components

Fresh flowers were separated from aerial parts, 80 g of flowers and 200 g of aerial parts were separately subjected to 4 hours hydro-distillation using simple distillation system. The hydro-distillation system consisted of 1L distillation flask containing the plant material in distilled water, connected with a condenser and then a 500 mL receiving flask filled with around 50 mL diethyl ether where the volatiles were collected in the receiving flask.

2.4. Gas Chromatography–Mass Spectrometry analysis

The GC-MS analysis was performed using Agilent Technologies 7890A gas chromatography system linked to Agilent Technologies 5975C inert mass spectrometry detector provided with Agilent Mass Hunter Acquisition software.

The GC system was equipped with Agilent Technologies 7693 auto-sampler (Agilent, United States), Agilent Technologies capillary column HP-5MS (Agilent, United States) filled with 5% phenyl methyl siloxane, (30 m x 0.25 mm I.D, 0.25 µm film thickness).

Injector auto-sampler injected a volume of 1 µL at 300 ºC. Helium gas was used as carrier gas with constant flow rate at 1.2232 mL min^{-1}, column head pressure was 22.231 psi. Oven temperature was programmed starting by initial temperature 90ºC for 1 min then increased by rate 8ºC/min to 205 ºC for 1 min then 5ºC/min to 250 ºC for 1 min and finally 8ºC/min to 300ºC for 30 min so that the total run time was 61.875 min. Electron energy was adjusted at 70 eV.

Identification of the peaks was carried out by matching their mass spectra with those present in Agilent Mass Hunter Acquisition software library. Further identification was achieved by comparing the data obtained with those reported in literature.

2.5. Antimicrobial activity

Antimicrobial and antifungal activities of the volatiles of both flowers and aerial parts of the plant were screened using the agar diffusion method according to guidelines of National Committee for Clinical Laboratory Standards (NCCLS), (2002). Samples were tested against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and fungi (*Candida albicans and Aspergillus flavus*). Bacterial and fungal cultures were obtained from Microbiology Dpartment of Faculty of Medicine, (Demerdash Hospital), Ain Shams University, Cairo, Egypt. Ketoconazole and gentamicin were used as standards antimicrobial agents.

One ml of 24 hours broth culture of each tested organisms was separately inoculated into 100 ml of sterile molten nutrient agar maintained at 45 ºC. The inoculated medium was mixed well and poured into sterile 10 cm diameter Petri-dishes, receiving 15 mL. Standards were weighed and dissolved up to concentration 20mg/ml in DMSO; Then volume of 100 µL of each of standard and the tested volatiles of both flowers and fresh aerial part were inserted in each well (6 mm diameter) followed by incubation at 37 ºC for 24 hours.

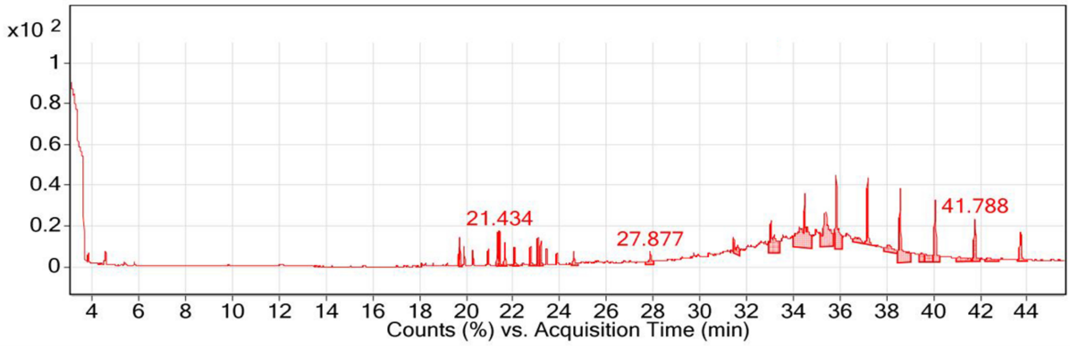

Figure 1: Gas chromatogram of *Carthamus lanatus* (L.) aerial parts

Table 1: Volatiles from *Carthamus lanatus* L. aerial parts

Rt (min)	Compound name	Rt (min)	Compound name
15.610	Decanoic acid, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-9aH-cyclopropa[3,4]benz[1,2-e]azulene-9,9a-diyl ester, [1aR-(1aα,1bβ,4aβ,7aα,7bα,8α,9β,9aα)]-	23.079	(1-Pentyloctyl)benzene
18.100	(1-Butylhexyl)benzene	23.204	(1-Butylnonyl)benzene
18.250	(1-Propylheptyl)benzene	23.442	(1-Propylheptadecyl) benzene
18.581	Methyl 6,8-octadecadiynoate	23.892	(1-Ethylundecyl)benzene
19.000	8-Octadecenal	24.611	(1-Methyldodecyl)benzene
19.201	(1-Methylnonyl)benzene	27.877	1-(4-Bromobutyl)-2-piperidinone
19.663	(1-Pentylhexyl)benzene	31.467	1-Heptacosanol
19.720	(1-Butylheptyl)benzene	33.056	Octatriacontyl pentafluoropropionate
19.907	(1-Propyloctyl)benzene	34.501	2-Octadecyloxyethanol
20.289	(1-Ethylnonyl)benzene	35.833	7-Hexylicosane
20.940	(1-Methyldecyl)benzene	37.191	Heneicosane
21.346	(1-Pentylheptyl)benzene	38.117	17-Pentatriacontene
21.434	(1-Butyloctyl)benzene	38.573	2-Methylicosane
21.659	(1-Propylnonyl)benzene	40.081	Octacosane
22.059	(1-Ethyldecyl)benzene	41.776	Tetratetracontane
22.754	(1-Methylundecyl)benzene	43.740	Heptacosane

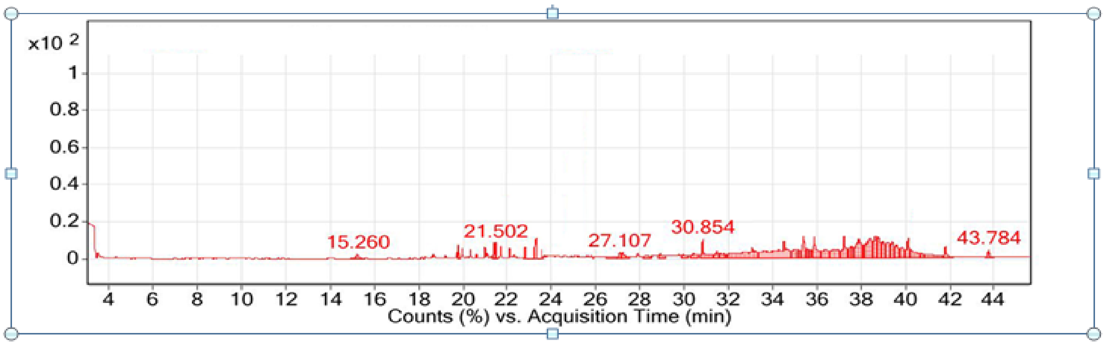

Figure 2: Gas chromatogram of *Carthamus lanatus* (L.) flowers

Table 2: Volatiles from *Carthamus lanatus* L. flowers

Rt (min)	Compound name	Rt (min)	Compound name
15.260	6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	22.822	(1-Methylundecyl)benzene
19.238	(1-Methylnonyl)benzene	27.107	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde
19.726	(1-Pentylhexyl)benzene	27.214	Propanoic acid, 2-methyl-, (dodecahydro-6a-hydroxy-9a-methyl-3-methylene-2,9-dioxoazuleno[4,5-b]furan-6-yl)methyl ester, [3aS-(3aα,6β,6aα,9aβ,9bα)]-
19.964	(1-Propyloctyl)benzene	27.914	tert-Hexadecanethiol
20.351	(1-Ethylnonyl)benzene	28.921	N-(2-Methylbutyl)undeca-(2E,4E)-diene-8,10-diynamide
20.614	Aromadendrene oxide-(1)	30.454	7-Methyl-Z-tetradecen-1-ol acetate
21.008	(1-Methyldecyl)benzene	30.848	Tributyl acetylcitrate
21.102	3-ethyl-3- hydroxyandrostan-17-one	33.081	Ethanol, 2-(octadecyloxy)-
21.402	(1-Pentylheptyl)benzene	35.890	7-Hexylicosane
21.496	(1-Butyloctyl)benzene	37.253	2,6,10-Trimethyltetradecane
21.721	(1-Propylnonyl)benzene	40.143	2-Methyleicosane
22.128	(1-Ethyldecyl)benzene	41.820	Tetratetracontane
22.322	Columbin	43.784	Heptacosane

Table 3: Comparison between volatiles isolated from Egyptian and Bulgarian *Carthamus lanatus L.*

Compound	B	E	Compound	B	E
Hydrocarbons			***Aromatics***		
Heptacosane	+	+	(1-Methylnonyl)benzene	-	+
Octacosane	+	+	(1-Pentylhexyl)benzene	-	+
Heneicosane	+	+	(1-Propyloctyl)benzene	-	+
7-Hexylicosane	-	+	(1-Ethylnonyl)benzene	-	+
17-Pentatriacontene	-	+	(1-Methyldecyl)benzene	-	+
2-Methylicosane	-	+	(1-Pentylheptyl)benzene	-	+
2,6,10-Trimethyltetradecane	-	+	(1-Butyloctyl)benzene	-	+
2-Methyleicosane	-	+	(1-Propylnonyl)benzene	-	+
3-Tetradecen-5-yne	+	-	(1-Ethyldecyl)benzene	-	+
Docosane	+	-	(1-Methylundecyl)benzene	-	+
Tricosane	+	-	(1-Butylhexyl)benzene	-	+
Tetracosane	+	-	(1-Propylheptyl)benzene	-	+
Pentacosane	+	-	(1-Butylheptyl)benzene	-	+
Hexacosane	+	-	(1-Pentyloctyl)benzene	-	+
Nonacosaner	+	-	(1-Butylnonyl)benzene	-	+
Triacontane	+	-	(1-Propylheptadecyl) benzene	-	+
Hentriacontane	+	-	(1-Ethylundecyl)benzene	-	+
Tetratetracontane	-	+	(1-Methyldodecyl)benzene	-	+
Aldehydes and ketones			Toluene	+	-
6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	-	+	Benzene isocyanate	+	-
Androstan-17-one, 3-ethyl-3-hydroxy-, (5α)-	-	+	Phenol-4,6-di-(1,1-dimethyl ethyl)-2-methyl	+	-

8-Octadecenal	-	+	Benzene-1,1_-(1,1,2,2 tetramethyl - 1,2-ethanediyl) bis 2,4-Diphenyl-4-methyl-1-pentene	+	-
1-Heptacosanol	-	+	***Terpenes***		
2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	-	+	α-Bisabolol	+	-
1-(4-Bromobutyl)-2-piperidinone	-	+	Caryophyllene oxide	+	-
2,5-Furandione-3-(1,1-dimethyl ethyl)	+	-	***Sulfur compounds***		
Nonanal	+	-	tert-Hexadecanethiol	-	+
Decanal	+	-	Dimethyl disulfide	+	-
Dodecanal	+	-	Methyl sulfonyl ethane	+	-
2,5-Cyclohexadiene-1,4-dione- bis(1,1-dimethyl ethyl)	+	-	1,2-Benzisothiazole	+	-
2,5-Cyclohexadiene-1-one-2,6-bis-(1,1-dimethyl ethyl)-4-ethylidene	+	-	Cycloctanoic sulfur	+	-
Ester and amides			***Alcohol***		
Propanoic acid, 2-methyl-, (dodecahydro-6a-hydroxy-9a-methyl-3-methylene-2,9-dioxoazuleno[4,5-b]furan-6-yl)methyl ester, [3aS-(3aα,6β,6aα,9aβ,9bα)]-	-	+	2-Octadecyloxyethanol	-	+
Decanoic acid, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetramethyl-5-oxo-9aH-cyclopropa[3,4]benz[1,2-e]azulene-9,9a-diyl ester, [1aR-(1aα,1bβ,4aβ,7aα,7bα,8α,9β,9aα)]-	-	+	***Acids***		
Tributyl acetylcitrate	-	+	2-Methyl butanoic acid	+	-
7-Methyl-Z-tetradecen-1-ol acetate	-	+	***Others***		
Columbin	-	+	Erucyclamide	+	-
Methyl 6,8-octadecadiynoate	-	+	Aromadendrene oxide-(1)	-	+
Octatriacontyl pentafluoropropionate	-	+	1,1,2,2-Tetrachloroethane	+	-
N-(2-Methylbutyl)undeca-(2E,4E)-diene-8,10-diynamide	-	+			

B: Bulgarian plant, E: Egyptian plant

The sensitivities were compared by measuring inhibition zones (in mm) with those of standards.

3. Results and discussion

3.1. Gas Chromatography–Mass Spectrometry analysis

The GC- MS qualitative analysis of volatiles yield by hydro-distillation of the fresh aerial parts of *Cartahmus lanatus* L. revealed the presence of 32 compounds, appeared in (**Figure 1).** These compounds belonged to different chemical classes (hydrocarbons, aldehydes and ketones, alcohols, esters, amides and different aromatic compounds). It is worth to mention that nine compounds were identified for the first time from genus *Carthamus*, namely, (1-Propyl-nonyl)benzene; (1-Ethyl-decyl)benzene; (1-Methyl-dodecyl) benzene; 1-Heptacosanol; Heneicosane; 2-Methylicosane; Octacosane; Tetratetracontane and Heptacosane.Moreover, twenty three of the revealed compounds were reported for the first time from family Asteraceae. Those compounds are: (1-Butylhexyl)benzene; (1-Propylheptyl)benzene; Methyl 6,8-octadeca diynoate; 8-Octadecenal; (1-Methylnonyl) benzene; (1-Pentylhexyl)benzene; (1-Butylheptyl)benzene; (1-Propyloctyl) benzene; (1-Ethylnonyl)benzene; (1-Methyldecyl)benzene; (1-Pentylheptyl) benzene; (1-Butyloctyl)benzene; (1-Methylundecyl)benzene; (1-Pentyloctyl)benzene; (1-Butylnonyl)benzene; (1-Propylheptadecyl) benzene; (1-Ethylundecyl) benzene; 1-(4-Bromobutyl)-2-piperidinone; Octatriacontyl pentafluoropropionate; 2-Octadecyloxy-ethanol; 7-Hexylicosane; 17-Pentatriacontene and Decanoic acid, 1,1a,1b,4,4a,5,7a,7b,8,9-decahydro-4a,7b-dihydroxy-3-(hydroxymethyl)-1,1,6,8-tetra-methyl-5-oxo-9aH-cyclopropa[3,4]benz[1,2-e]azulene-9,9a-diyl ester, [1aR-(1aα,1bβ,4aβ, 7aα, 7bα,8α,9β,9aα)]. The chemical composition of the volatiles of fresh aerial parts of *Cartahmus lanatus* L. are summarized in (**Table 1**).

Qualitative analysis the fresh flowers' volatiles showed the presence of 26 compounds of the same chemical classes as those present in the aerial parts (**Figure 2**). In addition, for the first time in *lanatus* species, three new compounds were figured out by this qualitative analysis: aromadendrene oxide; 3-ethyl-3- hydroxyandrostan-17-one and 7-Methyl-Z-tetradecen-1-ol acetate. Meanwhile, for the first time in genus *Carthamus*, eight compounds were reported:(1-Propylnonyl)benzene; (1-Ethyldecyl)benzene; tert-Hexadecanethiol; N-(2-Methylbutyl)undeca-(2E,4E)-diene-8,10-diynamide; 2,6,10-Trimethyltetradecane; 2-Methyleicosane; Tetratetracontane and Heptacosane. It is worth mentioning that, fifteen compounds were reported from family Asteraceae for the first time. Those are 6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one; (1-Methylnonyl) benzene; (1-Pentylhexyl)benzene; (1-Propyloctyl)benzene; (1-Ethylnonyl)benzene; (1-Methyldecyl)benzene; (1--Pentylheptyl)benzene; (1-Butyloctyl)benzene; Columbin; (1-Methylundecyl)benzene; 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde; Propanoic acid, 2-methyl-, (dodecahydro-6a-hydroxy-9a-methyl-3-methylene-2,9-dioxoazuleno[4,5-b]furan-6-yl)methylester,[3aS(3aα,6β,6aα,9aβ,9bα)]; Tributyl acetylcitrate; Ethanol, 2-(octadecyloxy) and 7-Hexylicosane. The chemical composition of the volatiles of fresh flowers of *Cartahmus lanatus* L. are summarized in (**Table 2**).

3.2.Comparative study between volatiles isolated from Egyptian and Bulgarian *Carthamus lanatus* L.

When it comes to the second comparative part of the study, it was easily observed that, there is a great difference between the volatiles' composition between the Egyptian *Carthamus lanatus* and the Bulgarian one. Only three compounds were in common between the two plants which were heptacosane, octacosane and heneicosane. Analysis of the obtained results showed that the volatiles composition of Egyptian plant was richer in ester and aromatic compounds, while that of the Bulgarian one was richer in hydrocarbons. The composition of the volatiles of both plants is summarized in (**Table 3**).

3.2. Antibacterial and Antifungal activities of volatiles

The biological screening of the volatiles of fresh aerial parts of Egyptian *Carthamus lanatus* revealed a potent antifungal activity against *Candida albicans,* even more potent than ketoconazole antifungal drug, but only a moderate antifungal activity against *Aspergillus flavus*. Regarding the antibacterial activity, the volatiles showed moderate activity against *Bacillus subtilis* only, with no activity against methicillin resistant *Staphylococcus aureus* (MARSA), *E.coli* and *K. pneumonia*. Gentamycin was used as reference standard antibacterial drug.

The antimicrobial activity results of volatiles of the fresh flowers showed potent antibacterial activity against MARSA, *E.coli* and *K. pneumonia* compared with gentamycin, while they showed moderate activity against *Bacillus subtilis*. The volatiles also showed good antifungal activity against both *Candida albicans* and *Aspergillus flavus*. The results of antimicrobial activity studies are summarized in (**Table 4).**

Table 4: Antimicrobial activity of volatiles from *Carthamus lanatus* L.

Diameter of inhibition zone (mm)				
Pathogenic microorganism	**Flower part**	**Aerial Part**	**Ketoconazole[a]**	**Gentamycin[b]**
Bacillus subtilis	12	9	N.A	26
Staphylococcus aureus (MARSA)	19	N.A	N.A	24.1
Klebsiella pneumoniae	20	N.A	N.A	21
Escherichia Coli	25	N.A	N.A	23
Candida albicans	18	24	20.3	N.A
Aspergillus flavus	15	8	17.1	N.A

[a]Antifungal standard, [b]Antibacterial standard

4. Conclusion

The obtained results from GC-MS analysis of the volatiles isolated from Egyptian *Carthamus lanatus* L. were remarkably different than those of the Bulgarian plant, with the appearance of 28 compounds for the first time from family Asteraceae, 12 compounds reported for the first time from genus *Carthamus*, in addition to the appearance of 3 compounds from *lanatus* species for the first time. Further studies are needed to justify those huge differences between the Egyptian and the Bulgarian *Carthamus lanatus* L. The antimicrobial screening of the volatiles isolated against different types of bacteria and fungi suggested the use of the volatiles of Egyptian plant as a promising antimicrobial candidate drug.

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