The lipid and volatile oil of the seed and aerial parts of *Onopordum alexandrinum* Boiss. growing in Egypt and their antioxidant activity

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Purpose
The study of the lipid of the seed and aerial parts of *Onopordum alexandrinum* Boiss., family Asteraceae, growing in Egypt led to the isolation and identification of the unsaponifiable and fatty acid fractions of both the seed and the aerial parts of the plant. Also, isolation of volatile oil of the seed and studying their antioxidant activity.

Subjects and methods
The constituents of the unsaponifiable fraction of the seed, aerial parts of the plant and volatile oil of the seeds were identified by Gas liquid chromatography (GLC) analysis. The radical scavenging effects of the tested extracts of both the seed and the aerial parts of the plant using DPPH free radicals.

Results
GLC analysis of the unsaponifiable fraction of the seeds indicated a mixture of hydrocarbons ranging from C_{13} to C_{28}; cholesterol, campasterol, stigmasterol, and β-sitosterol were also present. GLC analysis of the fatty acid methyl esters of the seeds indicated the presence of eight fatty acids. Also, GLC analysis of the unsaponifiable fraction of the aerial parts of the plant indicated a mixture of hydrocarbons ranging from C_{17} to C_{28}. Cholesterol, campasterol, stigmasterol, and β-sitosterol were also present. GLC analysis of the fatty acid methyl esters of the aerial parts indicated the presence of nine fatty acids. Sixteen compounds were identified by GLC analysis represented 77.97% of the total composition of the oil of the seeds.

Conclusion
The unsaponifiable fractions of both the seed and the aerial parts of the plant and volatile oil showed a strong antioxidant activity, whereas the fatty acid fractions showed a moderate antioxidant activity.

Keywords:
antioxidant activity, *Asteraceae*, fatty acids, *Onopordum alexandrinum*, unsaponifiable fraction, volatile oil

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Introduction

The family *Asteraceae* is one of the largest families of flowering plants, with approximately 1100 genera and 2500 species [1]. In Egypt, there are 97 genera including 230 species [2]. *Onopordum* is a genus of about 40 species. In Egypt, there are only two species of *Onopordum*: *O. alexandrinum* and *O. ambiguum* [2,3]. A review of the literature indicated that several plants of the genus *Onopordum* have antimicrobial and antitumor activities [4]. *O. alexandrinum* is used as an expectorant, for healing wounds, and as a treatment of skin cancers [5,6].

Preliminary phytochemical investigations of the seeds of *O. alexandrinum* growing in Egypt using standard procedures have shown that it contains terpenes, flavonoids, coumarins, sterols, and volatile oils.

There is very little information on the phytoconstituents of the seeds of *O. alexandrinum*. Therefore, in this work, the lipid and volatile oil of the seeds of the plant and the antioxidant activities of both the lipid and the volatile oil constituents are studied.

Subjects and methods

Plant material

O. alexandrinum Boiss. was collected from Bourg El-Arab near Alexandria in May 2010. The plant was identified by Dr M. El-Gebaly and Dr S. El-Kawashty, who are taxonomists at the National Research Centre (Cairo, Egypt) to whom the authors are very grateful. The seeds and aerial parts of the plant were air dried and ground into a fine powder. A voucher specimen was kept in the herbarium of National Research Center.

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Reagent and solvent

6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox; Aldrich Chemical Co., Germany), 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma Chemical Co., USA), and methanol (HPLC grade) were used.

Apparatus and techniques

Gas liquid chromatography (GLC, Hewellett HP-6890 series, USA) was carried out. GLC analyses were carried out under the following conditions.

For the unsaponifiable matter

Column: HP-1 (methyl siloxane) 30 m length/0.53 × 2.65 μm, temperature program: initial temperature 60°C, initial time 2 min, program rate 10°C/min, final temperature 280°C, final time 30 min, injection temperature 260°C, detector (FID), $T = 300$°C, flow rate of carrier gas N_2: 30 ml/min, H_2: 35 ml/min, air: 30 ml/min.

For fatty acids

Methyl esters of fatty acids were analyzed on HP-6890 GC.

Column: HP-5 (phenyl methyl siloxane) 30 m length/0.32 × 2.25 μm, temperature program: initial temperature 60°C, initial time 2 min, program rate 8°C/min, final temperature 270°C, final time 10 min, injection temperature 270°C, detector (FID), $T = 300$°C, flow rate of carrier gas N_2: 30 ml/min, H_2: 35 ml/min, air: 30 ml/min.

For the volatile oil

Column: HP-INNOWAX-polyethylene glycol, length: 30 m, diameter: 250 μm, film thickness: 0.15 μm. Oven temperature: initial temperature 70°C, initial time 2 min. Ramps: rate 3°C/min, final temperature 190°C, final time 5 min, injection temperature 250°C, detector (FID), $T = 260$°C, flow rate of carrier gas N_2: 33 ml/min, H_2: 35 ml/min, air: 330 ml/min.

Preparation of the lipid matter of the seeds

Approximately 300 g of the air-dried powdered seeds of *O. alexandrinum* Boiss. were extracted with petroleum ether (40–60°C). The purified extract was evaporated and the residue (3.349 g) was dissolved in boiling acetone (100 ml) and cooled; however, no precipitate was found. The acetone-soluble fraction was saponified (N/2 alc. KOH) and the unsaponifiable matter (1.21 g) was separated. The fatty acid mixture released was extracted and methylated (methanol, 4.5% HCl). Samples of the isolated unsaponifiable fraction and the methyl esters of fatty acids were subjected to GLC analyses [7].

Preparation of the lipid matter of the aerial parts

Approximately 350 g of the air-dried powdered aerial parts of *O. alexandrinum* Boiss. were extracted with petroleum ether (40–60°C). The purified extract was evaporated and the residue (4.95 g) was dissolved in boiling acetone (100 ml) and cooled; however, no precipitate was found. The acetone-soluble fraction was saponified (N/2 alc. KOH) and the unsaponifiable matter (1.32 g) was separated. The fatty acid mixture released was extracted and methylated (methanol, 4.5% HCl). Samples of the isolated unsaponifiable fraction and the methyl esters of fatty acids were subjected to GLC analyses [7].

Preparation of the volatile oil

Approximately 300 g of the dried powdered seeds of *O. alexandrinum* were macerated with three-fold their weight with petroleum ether (40–60°C) at room temperature for 48 h. The maceration was repeated twice. The petroleum ether extract was evaporated and then subjected to water distillation for about 3 h until no more volatile oil could be distilled. The oil was extracted from the aqueous layer by shaking with ether, which was evaporated after dehydration over anhydrous sodium sulfate. A yellowish green oil was obtained (0.59 g). The volatile oil was subjected to GLC analysis, and the compounds were identified by comparison of their relative retention times with the available authentics [8].

Antioxidant activity

Determination of scavenging effect on DPPH radicals

The effect of the plant extracts on DPPH was studied using the modified method described by Chen and Ho [9]. The decrease in the absorbance of the DPPH solution at 516 nm after the addition of the sample (plant materials) was measured in a glass cuvette. An aliquot of a 0.1 ml M. methanol solution of DPPH was mixed with the methanolic solution of the sample, so that the relative concentration of plant materials versus the stable radical in the cuvette was 0.13; then the solution with the tested sample was shaken vigorously. The absorbance was determined at the start and 30 min after being kept in the dark against a blank of methanol without DPPH. All tests were run in duplicate and averages were calculated [10]. The antioxidative activities of these samples were compared with Trolox as follows:

$$\%\ \mathrm{RSA} = 100\,\% \times \frac{\text{Absorbance of blank}_{516\,\mathrm{nm}} - \text{Absorbance of sample}_{516\,\mathrm{nm}}}{\text{Absorbance of blank}_{516\,\mathrm{nm}}},$$

where RSA is the radical scavenging activity.

Results

Unsaponifiable fraction of the seeds

GLC analysis for the unsaponifiable fraction of the seeds of *O. alexandrinum* indicated a mixture of hydrocarbons ranging from C_{13} to C_{28}, in which C_{23} (45.39%) was the main hydrocarbon. Also, cholesterol, campasterol, stigmasterol, and β-sitosterol were present, in which stigmasterol was the main sterol (4.245%) (Table 1).

Unsaponifiable fraction of the aerial parts

GLC analysis of the unsaponifiable fraction of the aerial parts of *O. alexandrinum* indicated a mixture of hydrocarbons ranging from C_{17} to C_{28}, in which C_{23} (5.8%) was the main hydrocarbon. Also, cholesterol, campasterol,

stigmasterol, and β-sitosterol were present, in which β-sitosterol was the main sterol (22.98%) (Table 2).

Table 1 Gas liquid chromatography analysis of the unsaponifiable fraction of the seeds of *Onopordum alexandrinum* Boiss.

Peak number	RRT	Relative (%)	Constituents
1	9.7323	5.0900	C_{13} n-tridecanoic
2	20.9030	8.54352	C_{20} n-cosane
3	22.5320	2.3251	Unidentified
4	25.104	45.3906	C_{23} n-tricosane
5	27.0301	2.3976	C_{25} n-pentacosane
6	28.5440	6.8787	C_{27} n-heptacosane
7	29.7990	14.2544	C_{28} n-octacosane
8	30.3270	4.1200	Unidentified
9	31.0600	1.3122	Unidentified
10	32.3860	2.2385	Cholesterol
11	34.6970	0.9006	Campasterol
12	35.3840	4.2453	Stigmasterol
13	37.1420	2.3030	β-Sitosterol

Identified sum=92.2426%.
Unidentified sum=7.7573%.

Table 2 Gas liquid chromatography analysis of the unsaponifiable fraction of the aerial parts of *Onopordum alexandrinum* Boiss.

Peak number	RRT	Relative (%)	Constituents
1	16.776	2.31798	C_{17} n-heptadecane
2	18.285	2.96905	C_{18} n-octadecane
3	19.188	2.90457	C_{19} n-nonadecane
4	20.372	4.01023	Unidentified
5	21.147	5.62529	C_{20} n-cosane
6	21.995	2.23110	Unidentified
7	22.629	2.49529	C_{21} n-uncosane
8	24.027	2.74172	C_{22} n-dodecosane
9	25.041	5.68700	C_{23} n-tricosane
10	25.908	1.88408	C_{24} n-tetracosane
11	26.453	1.82466	C_{25} n-pentacosane
12	27.264	1.25765	Unidentified
13	27.688	2.13184	C_{26} n-hexacosane
14	28.524	2.09122	C_{27} n-heptacosane
15	29.318	3.43696	C_{28} n-octacosane
16	30.280	1.50102	Unidentified
17	31.862	3.09573	Cholesterol
18	34.624	12.13007	Campasterol
19	35.395	16.84430	Stigmasterol
20	37.197	22.29808	β-Sitosterol

Identified sum=90.4778%.
Unidentified sum=9%.
RRT, Relative to retention time of palmitic acid $C_{16\ (0)(17.49\ min)}$.

Table 3 Gas liquid chromatography analysis of the fatty acid methyl esters of the seeds of *Onopordum alexandrinum* Boiss.

Peak number	RRT	Relative (%)	Constituents
1	10.774	1.5590	C_9 nonanoic
2	11.950	6.3598	C_{10} decanoic
3	13.596	1.1072	Unidentified
4	15.419	0.2302	C_{12} lauric acid
5	18.043	20.4462	$C_{16\ (0)}$ palmitic acid
6	18.258	2.3582	Unidentified
7	20.202	43.9244	$C_{18\ (1)}$ oleic acid
8	20.325	8.3724	$C_{18\ (2)}$ linoliec acid
9	20.504	2.9304	$C_{18\ (3)}$ linolenic acid
10	22.141	3.5150	Unidentified
11	23.724	4.5210	Unidentified
12	24.657	3.3866	$C_{20\ (4)}$ eicosatetraenoic acid

Identified sum=87.2095%.
Unidentified sum=11.4015%.
RRT, Relative to retention time of palmitic acid $C_{16\ (0)(17.49\ min)}$.

Table 4 Gas liquid chromatography analysis of the fatty acid methyl esters of the aerial parts of *Onopordum alexandrinum* Boiss.

Peak number	RRT	Relative (%)	Constituents
1	12.774	1.5452	C_{10} decanoic
2	15.425	1.81010	C_{12} lauric acid
3	18.002	27.52748	C_{16} palmitic acid
4	18.575	3.1053	Unidentified
5	20.019	31.14147	$C_{18\ (0)}$ stearic acid
6	20.123	18.72731	$C_{18\ (1)}$ oleic acid
7	20.203	5.85568	$C_{18\ (2)}$ linoliec acid
8	20.511	0.52686	$C_{18\ (3)}$ linolenic acid
9	22.164	4.2525	Unidentified
10	24.035	1.5010	Unidentified
11	24.589	1.84017	$C_{20\ (4)}$ eicosatetraenoic acid
12	26.652	2.0012	Unidentified

Identified sum=88.9742%.
Unidentified sum=10.8699%.
RRT, Relative to retention time of palmitic acid $C_{16\ (0)(17.49\ min)}$.

Fatty acid fraction of the seeds

GLC analysis of the fatty acid methyl esters of the seed of the plant indicated that the presence of eight fatty acids represented 87.21% of the total acids, in which oleic acid $C_{18(1)}$ was the main acid (43.924%) as shown in Table 3.

Fatty acid fraction of the aerial parts

GLC analysis of the fatty acid methyl esters of the aerial parts of the plant indicated that the presence of nine fatty acids represented 88.97% of the total acids, in which stearic acid $C_{18(0)}$ was the main acid (31.14%) as shown in Table 4.

Discussion

GLC analysis of the unsaponifiable fraction of the seeds of *O. alexandrinum* indicated a mixture of hydrocarbons ranging from C_{13} to C_{28}, in which C_{23} (45.39%) was the main hydrocarbon. Also, cholesterol, campasterol, stigmasterol, and β-sitosterol were present, in which stigmasterol was the main sterol (4.245%) (Table 1).

Also, GLC analysis of the unsaponifiable fraction of the aerial parts of the plant indicated a mixture of hydrocarbons in which C_{23} (5.8%) was the main hydrocarbon. Also, a mixture of sterol compounds was present in which β-sitosterol was the main sterol (22.98%), Table 2. Identification of the compounds was carried out by comparison of their retention time with the available reference compounds.

GLC analysis of the fatty acid methyl esters of the seed of the plant indicated that the presence of eight fatty acids represented 87.21% of the total acids, in which oleic acid ($C_{18(1)}$, 43.924%) and palmitic acid (C_{16}, 20.44%) were the main acids (Table 3). GLC analysis of the fatty acid methyl esters of the aerial parts of the plant indicated that the presence of nine fatty acids represented 88.97% of the total acids, in which stearic acid $C_{18(0)}$ was the main acid (31.14%) as shown in Table 4.

The constituents of the volatile oil obtained from the seeds of *O. alexandrinum* were identified using GLC analysis. Sixteen compounds were identified, which represent 77.97% of the total composition of the oil, in

Table 5 Constituents identified in the volatile oil of the seeds of *Onopordum alexandrinum* Boiss.

Identified compounds	RRT	Relative (%)
Linolyl oxide	9.76	0.679
Linalool	10.68	0.187
Pinocarvone	12	0.736
Terpinene-4-ol	12.9	2.148
α-Terpinene	13.5	2.358
Trans-carneol	14.4	3.328
Martenol	14.61	5.666
α-Terpineol	14.86	3.011
Carvone	15.21	3.741
Trans-carveol	15.81	16.097
	16.26	0.531
	16.40	1.484
Pipertone	16.63	3.694
Bornyl acetate	16.76	2.404
	17.26	2.278
Carvacrol	17.63	9.134
	18.01	4.207
	18.43	6.229
	18.63	3.662
	18.86	0.658
	19.35	2.048
	19.53	0.370
α-Cubebene	21.05	7.230
β-Cubebene	23.31	5.476
	23.55	0.570
δ-Cadinene	26.28	12.086

Identified sum = 77.97%.
Unidentified sum = 22.03%.

Table 6 Radical scavenging effect of samples on DPPH free radical

	Absorbance 516 nm/reaction period (min)[a]		
Tested compounds	10	30	RSA (%)
Trolox	0.025	0.026	95.07
Unsap. (s)	0.065	0.066	79.18
Unsap. (p)	0.075	0.079	82.83
Fatty acids (s)	0.182	0.189	64.02
Fatty acids (p)	0.118	0.125	62.7
Volatile oil	0.091	0.090	81.63

Fatty acids (p), fatty acid methyl esters of the aerial parts of the plant; fatty acids (s), fatty acid methyl esters of the seeds of the plant; RSA, radical scavenging activity; unsap. (p), unsaponifiable fraction of the aerial parts of the plant; unsap. (s), unsaponifiable fraction of the seeds.
[a]The absorbance reading at each reaction period is the mean of two measurements.

which carvacrol (9.134%), δ-cadinene (12.086%), and *trans*-carveol were the main constituents (Table 5).

The radical scavenging effects of the tested extracts of both the seed and the aerial parts of the plant using DPPH free radical were also determined (Table 6). The unsaponifiable fractions of both the seed and the aerial parts of the plant and volatile oil showed a strong antioxidant activity, whereas the fatty acid fractions showed a moderate antioxidant activity.

Conclusion

The bioactive constituents of herb and seeds of *O. alexandrinum* were evaluated for their biological activity. The unsaponifiable fractions of both the seed and the aerial parts of the plant and the volatile oil showed a strong antioxidant activity, whereas the fatty acid fractions showed a moderate antioxidant activity. Growing awareness of the protective functions of thistles highlights the need to conserve and manage them sustainably. Thus, *O. alexandrinum* is a very promising medicinal plant and a good candidate for further detailed and specific phytochemical and pharmacological studies.

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Conflicts of interest

There are no conflicts of interest.

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