Chemical composition and antimicrobial activity of volatile constituents from the roots, leaves, and seeds of *Arctium lappa* L. (*Asteraceae*) grown in Egypt

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Background and objective
As no literature was traced dealing with the volatile constituents of the leaves or the seeds of *Arctium lappa* L., it was deemed of interest to carry out a gas chromatography/mass spectrometry (GC/MS) analysis and antimicrobial activity study of the volatile constituents of roots, leaves, and seeds of the plant grown in Egypt.

Materials and methods
The volatile constituents of the roots, leaves, and seeds were analyzed by GC/MS. The antimicrobial activity was tested using the agar well diffusion technique.

Results and conclusion
GC/MS of the volatile constituents from the leaves showed 19 identified compounds, the major being caryophyllene oxide (54.2%), followed by β-elemene (6.2%) and β-costol (4.0%). Analysis of the volatile constituents of the roots revealed 14 identified compounds, the major being caryophyllene oxide (51%), followed by aromadendrene (16%) and isoaromadendrene epoxide (6.4%). Analysis of the volatile constituents of the seeds revealed 22 identified compounds, the major being *E*-citral (28.8%), followed by geraniol (20.3%) and *Z*-citral (9.5%). The volatile constituents of the leaves and roots exhibited moderate antimicrobial activity against bacteria and significant antifungal activity, in comparison with the standards used, whereas the volatile constituents of the seeds showed moderate antimicrobial activity against bacteria and fungi.

Keywords:
antibacterial, antifungal, *Arctium lappa* L., leaves, roots, seeds, volatile constituents

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Introduction

Arctium lappa L. or burdock (*Asteraceae*) is generally considered a safe and edible food product. Burdock root is commonly used as food in Asia [1,2] and is known in Japan as Gobo [3]. Studies on the biological activities of extracts from different organs of burdock and compounds isolated thereof were carried out including antipyretic, antimicrobial, diuretic, diaphoretic, hypoglycemic [4], in-vitro antioxidant [5–11] anti-inflammatory, antihepatotoxic [12,13], antiulcer [14], antimutagenic [15,16], and antitumour activities [17–19]. The plant was used abroad in many pharmaceutical preparations such as Flor-Essence and Essiac, which were suggested to improve the quality of life and prolong survival in cancer patients [20]. In our previous studies [21,22] on this plant cultivated in Egypt, its phytoconstituents, tissue culture products, and certain biological activities were evaluated.

A study on the volatile constituents from *A. lappa* L. root [23] revealed that the hydrocarbon fraction constituted 80% of the total volatiles, whereas another study [24] showed 63 identified compounds, methyl linolenate being the major compound. No literature was traced dealing with the volatile constituents of the leaves or the seeds. In the present work, the volatile constituents of this plant grown in Egypt were studied.

Materials and methods

Plant material

The plant *A. lappa* L. was cultivated in the experimental station, Faculty of Pharmacy, Cairo University, from the seeds kindly offered to Dr E.A. Aboutabl by colleagues at the Botanical Garden, Bohn, Germany. Voucher specimens were deposited at the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, Cairo University. The leaves and seeds were collected in July during the fruiting stage, whereas the roots were collected after the first year of growth and cut into small pieces.

Bacterial and fungal strains

Gram-negative bacteria: *E. coli* (NCT-10410); gram-positive bacteria: *Bacillus subtilis* (NCIB); and fungi: *Aspergillus niger* (ferm-Bam C-21) and *Candida albicans* were kindly offered by Fermentation, Biotechnology, and Applied Microbiology Center, Al Azhar University, Cairo.

Preparation of volatile constituents

A weight of 500 g of fresh roots, leaves, and seeds, respectively, were separately subjected to hydrodistillation [25]; they yielded (after drying over anhydrous sodium sulfate) 0.06, 0.1, and 0.2% v/w, respectively, and were stored at 4–6°C.

GC/MS analysis

The volatile components isolated from roots, leaves, and seeds were subjected to GC/MS analysis on Trace GC 2000 (Thermo) coupled with Finnigan mass spectrophotometer (SSQ7000) Frankfurt, Germany. Column: DB-5 (5% phenyl methyl polysiloxane); internal diameter: 0.25 mm; carrier gas: helium; flow rate: 1 ml/min; injection mode: splitless; temperature programming: 40°C at 0–5°C/min, then 40–140°C at 5°C/min, then 140–260°C at 6°C/min; total run time 45 min; scan mass range: 40–500 m/z. The identified components, their retention times, Kovat's indices, and mass spectral data are tabulated in Table 1. The components were identified by comparing their retention times, Kovat's indices, and mass fragmentation patterns with those of available libraries and standard references [26,27].

Antimicrobial activity

Antimicrobial activity was tested using the agar well diffusion technique [28]. Nutrient agar was used for inoculation of bacteria, whereas Dox media were used for inoculation of fungi. Each volatile oil in 50 μl aliquots were separately inoculated into wells, each of 1 cm diameter. The plates were incubated at 37°C for 24 h for bacteria and at 25°C for fungi. Chloramphenicol (Miphenicol; Misr Co., Cairo, Egypt) and cephalexin (Keflex; CEPH International

Table 1 GC/MS analysis of the volatile components from the roots, leaves, and seeds of *Arctium lappa* L. cultivated in Egypt

No.	Compound name	Molecular weight	Formula	KI	Base peak	Major peaks	Relative area %		
							Leaves	Roots	Seeds
1	α-Pinene	136	$C_{10}H_{16}$	939	93	92,77,53,41	0.50	0.09	–
2	β-Myrcene	136	$C_{10}H_{16}$	990	41	93,79,69,53	–	–	2.82
3	Limonene	136	$C_{10}H_{16}$	1029	68	93,79,67,53	0.23	–	0.03
4	Linalool	140	$C_{10}H_{18}O$	1096	71	121,93,55,43	–	–	1.12
5	Nonanal	142	$C_9H_{18}O$	1100	41	98,82,70,41	0.72	–	0.23
6	Carvomenthone	154	$C_{10}H_{18}O$	1208	82	137,110,95,39	0.11	0.03	–
7	Geraniol	154	$C_{10}H_{18}O$	1252	41	93,84,69,53	–	–	20.28
8	Thymol	150	$C_{10}H_{14}O$	1290	135	115,107,91,77	–	–	0.26
9	*Z*-citral	152	$C_{10}H_{16}O$	1318	198	135,123,83,69	–	–	9.45
10	*E*-citral	152	$C_{10}H_{16}O$	1341	198	134,123,83,69	–	–	28.84
11	Tetradecane	196	$C_{14}H_{28}$	1377	41	111,83,69,55	0.75	–	–
12	β-Elemene	204	$C_{15}H_{24}$	1390	204	107,81,67,41	6.16	–	3.02
13	Aromadendrene	204	$C_{15}H_{24}$	1441	41	161,105,91,79	–	16.00	0.76
14	*Trans*-β-farnesene	204	$C_{15}H_{24}$	1456	69	133,120,93,79	0.10	0.11	–
15	Pentadecane	212	$C_{15}H_{32}$	1500	57	99,85,71,43	1.43	–	–
16	γ-Cadinene	204	$C_{15}H_{24}$	1513	161	134,119,105,91	1.70	2.79	0.33
17	Caryophyllene oxide	220	$C_{15}H_{24}O$	1583	41	121,109,93,79	54.18	51.07	0.15
18	β-copaen-4α-ol	220	$C_{15}H_{24}O$	1590	159	131,118,91,41	2.06	1.69	0.43
19	Isoaromadendrene epoxide	220	$C_{15}H_{24}O$	1641	133	105,91,69,41	–	6.43	–
20	β-costol	220	$C_{15}H_{24}O$	1767	41	121,105,91,79	4.03	1.98	1.36
21	Nonadecane	268	$C_{19}H_{40}$	1900	57	99,85,71,43	1.77	–	–
22	Methyl palmitate	270	$C_{17}H_{34}O_4$	1921	74	87,143,227,75	–	–	1.59
23	Eicosane	282	$C_{20}H_{42}$	2000	57	99,85,71,43	1.48	0.35	0.34
24	Methyl oleate	296	$C_{19}H_{36}O_2$	2087	55	264,222,97,69	–	–	2.58
25	Octadecanoic acid methyl ester	298	$C_{19}H_{38}O_2$	2090	74	225,143	–	–	2.23
26	Ethyl oleate	310	$C_{20}H_{38}O_2$	2180	55	264,222,97,69	–	–	0.27
27	Docosane	310	$C_{22}H_{46}$	2200	57	99,85,71,43	0.64	–	–
28	Tetracosane	338	$C_{24}H_{50}$	2400	57	99,85,71,43	1.27	0.09	–
29	Pentacosane	352	$C_{25}H_{52}$	2500	57	99,85,71,43	0.09	0.19	0.09
30	Hexacosane	366	$C_{26}H5_4$	2600	57	99,85,71,43	2.67	1.41	2.32
31	Heptacosane	380	$C_{27}H_{56}$	2700	57	99,85,71,43	0.46	0.19	–
32	Squalene	410	$C_{30}H_{50}$	2790	69	149,121,95,81	–	–	1.50

Corporation, Cairo, Egypt), and fluconazole (Flucoral; SEDICO Pharmaceutical Co., Cairo, Egypt) and amikacin were used as reference standards.

Results

Analysis of the volatile components from the leaves (Table 1) showed 19 identified compounds, representing 80.4% of the total composition. The identified fraction had four oxygenated compounds contributing 61% and 15 unoxygenated compounds contributing 19.3%. The major compound was caryophyllene oxide (54.2%), followed by β-elemene (6.2%) and β-costol (4.0%). Analysis of the volatile oil from the roots (Table 1) showed 14 identified compounds, representing 85.4% of the total composition. The identified fraction had five oxygenated compounds contributing 61.2% and nine unoxygenated compounds contributing 21.2%. The major compound was caryophyllene oxide (51.1%), followed by aromadendrene (16%) and isoaromadendrene epoxide (6.4%). Analysis of the volatile oil from the seeds (Table 1) showed 22 identified compounds, representing 80.4% of the total composition. The identified fraction had 13 oxygenated compounds (representing 68.1%) and nine unoxygenated compounds (representing 12.4%). The major compound was *E*-citral (28.8%), followed by geraniol (20.3%) and *Z*-citral (9.5%). From the data in Table 2 and Figure 1, it was concluded that the volatile constituents of the leaves and roots showed moderate antimicrobial activity against bacteria and higher antifungal activity in comparison with the standards used. The volatile constituents of the seeds show moderate antimicrobial activity against bacteria and fungi. The volatile constituents of the leaves and the roots show minimum inhibitory concentration (Table 3) lesser than that of the standard, flucoral.

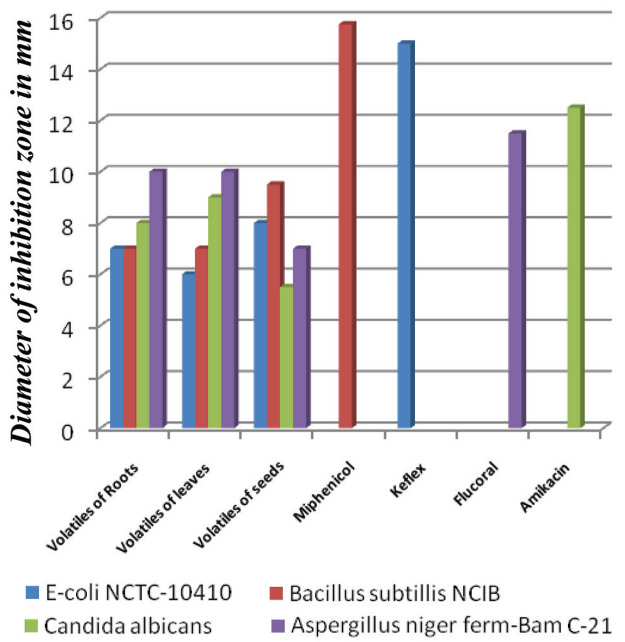
Table 2 Antimicrobial activity of volatile constituents of *Arctium lappa* L. roots, leaves, and seeds

Volatile components	Test organism (diameter of inhibition zone in mm)			
	Gram-negative bacteria	Gram-positive bacteria	Fungi	
	E-coli NCTC-10410	*Bacillus subtillis* NCIB	*Candida albicans*	*Aspergillus niger* ferm-Bam C - 21
Volatiles of roots (50 μl)	7	7	8	10
Volatiles of leaves (50 μl)	6	7	9	10
Volatiles of seeds	8	9.5	5.5	7
Miphenicol (50 μl of 1 mg/ml solution)	–	15.75	–	–
Keflex (50 μl of 1 mg/ml solution)	15	–	–	–
Flucoral (50 μl of 1 mg/ml solution)	–	–	–	11.5
Amikacin (50 μl of 1 mg/ml solution)	–	–	12.5	–

Discussion and conclusion

The percentages of the different classes of volatile constituents from *A. lappa* leaves, roots, and seeds are compiled in (Table 4). No literature was found on the composition of volatile constituents of *A. lappa* L. leaves and seeds. The available literature on the volatile constituents of *A. lappa* roots [23] showed that the total composition comprised 80% hydrocarbons. In this study, the volatile constituents from the root of this plant cultivated in Egypt comprised 21.2% hydrocarbons. The volatile constituents of the leaves and the roots showed caryophyllene oxide as a major component, representing 54.2 and 51.1%, respectively. In addition, the major volatile constituent of the seeds was *E*-citral (28. 8%). In total, oxygenated compounds represent over 60% of the volatile constituents from the three organs of the plant. There is a big difference in the volatile constituents of the root of *A. lappa* grown in Egypt, which may be attributed to the environmental conditions. This is the first report on the volatile constituents of *A. lappa* L. grown in Egypt. The antimicrobial activity of the volatile

Figure 1



Antimicrobial activity of volatile constituents of *Arctium lappa* L. roots, leaves, and seeds.

Table 3 Minimum inhibitory concentration of the volatile constituents of *Arctium lappa* L. roots, leaves, and seeds

Test material	Minimum inhibitory concentrations in μl/ml			
	Gram-negative bacteria	Gram-positive bacteria	Fungus	
	E-coli NCTC-10410	*Bacillus subtillis* NCIB	*Candida albicans*	*Aspergillus niger* ferm-Bam C-21
Volatiles of roots	1000	500	250	250
Volatiles of leaves	950	500	250	200
Volatiles of seeds	500	450	1000	1000
Miphenicol	–	65	–	–
Keflex	150	–	–	–
Flucoral	–	–	–	1000
Amikacin	50	–	75	–

Table 4 Percentages of different classes of identified volatile constituents of *Arctium lappa* L. leaves, roots, and seeds

Components	% Leaves	Roots	Seeds
Hydrocarbons	19.25	21.22	11.21
Alcohols	6.09	3.67	23.45
Aldehydes	0.72	–	38.52
Esters	–	–	6.51
Oxides	54.18	57.50	0.15
Total oxygenated compounds	60.99	61.17	68.63

constituents of this plant cultivated in Egypt was found to be significant. The volatile constituents of the leaves and roots showed higher antifungal activity, because of higher percentages of caryophyllene oxide.

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Conflicts of interest

There are no conflicts of interest.

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