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Volatile and lipid analyses by gas chromatography/mass spectrometry and nutraceutical potential of edible wild *Malva aegyptiaca* L. (Malvaceae)

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Abstract
Volatile and lipid chemical compositions, and nutritional and antioxidant properties of *Malva aegyptiaca*, an edible wild plant largely distributed in North Africa, were investigated. Forty-nine compounds of volatiles were identified showing large qualitative and quantitative differences during three phenological stages. The flowering stage was characterized by the presence of a high number of terpenic compounds, among them dillapiole was found to be the major one (55.15%). The nutrient composition of leaves and fruits was investigated in the present work. Fruits' lipidic fraction was characterized by its high level of linoleic acid (n-6) (36.17%). Interestingly, leaves' lipidic fraction was characterized by its very high content of camphor (43.69%) and by its relatively high content of linoleinic acid (n-3) (14.69%). Furthermore, our results showed that the phenolic contents varied from 352 to 404 mg gallic acid equivalent/g ethanolic and acetonic extracts, respectively. These extracts revealed interesting antioxidant activities including free radical scavenging activity ($EC_{50} = 0.38–0.57$ mg/ml) and reducing power ($EC_{50} = 0.12–0.18$ mg/ml).

Keywords: Malava aegyptiaca, *wild edible, volatiles, nutrients, lipids, antioxidant*

Introduction

A number of studies have focused on phytochemical characterization and evaluation of biological properties of various plants. In fact, many compounds isolated from plants have exhibited a wide spectrum of biological activities and have received particular attention as potential natural agents for human health and nutrition. For example, natural antioxidants from fruits and medicinal plants have recently become highly sought because of their benefits to human health. The regular intake of natural antioxidants contribute to the protection against cancer, cardiovascular disease, diabetes, and other ageing-related diseases by reducing oxidative stress (Kris-Etherton et al. 2002). Several fruits and herbs were described for their antioxidant properties, which are mainly attributed to a variety of active natural antioxidants including flavonoids, polyphenols, alkaloids, anthocyanins, terpenoids, carotenoids and vitamins (Espin et al. 2007). This explains the growing importance of finding natural antioxidants protecting humans from oxidative stress damage. Furthermore, essential fatty acids are required for normal growth and good health. Particularly, the omega-3 fatty acids have demonstrated cardio-protective effects (Juturu 2008). In fact, essential fatty acid consumption decreases blood pressure, triglyceride levels and inflammatory markers, improves endothelial function, reduces platelet aggregation and vasoconstriction, and decreases the risk of sudden cardiac death (Juturu 2008). Plants that are able to synthesize these compounds could be a source

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of these fatty acids in our diet. Moreover, edible wild plants might also be important sources of minerals, beneficial to human health (Guil et al. 1998).

Malva is a genus of about 25–30 species of herbaceous plants in the *Malvaceae* family. The genus is widespread throughout the temperate, subtropical and tropical regions of Africa, Asia and Europe. *Malva aegyptiaca* L. (vernacular name: khoubbiza) is a wild plant of the mallow family, widespread in North Africa and edible for the local population. *M. aegyptiaca* is an annual species, glabrous and weakly branched. Its stems are prostrate and its length usually does not exceed 30 cm. The leaves are linear, weakly toothed and with long petioles. The white flowers are small and appear grouped. The fruit is clearly marked by projecting rays. This plant is rarely found in steppe and appears mostly as nitratophile. It is found in several regions from the north to the south of Tunisia. This species is consumed cooked in boiling water and then mixed with a spicy sauce and couscous. Furthermore, this plant also appears to have therapeutic properties (Chaieb and Boukhriss 1998, Le Floc'h and Boulos 2008).

Many chemical compounds have been isolated and identified from *Malva sylvestris*, such as 8-hydroxyflavonoid glucuronides and malonated anthocyanins (Takeda et al. 1989, Billeter et al. 1991). Furthermore, the extracts of different parts of *M. sylvestris* were compared for their antioxidant properties and chemical composition (Barros et al. 2010).

M. aegyptiaca is widely and largely distributed and generally underutilized in culinary habits. To our knowledge, the physicochemical and biological properties of this plant have not been reported in the literature. Therefore, we aimed to increase the knowledge about the nutritional properties (protein, carbohydrate, lipid, and minerals) of *M. aegyptiaca* leaves and fruits. Analysis of volatile compounds at different phenological stages and lipidic components by gas chromatography (GC) and GC/mass spectrometry (MS) techniques was also carried out. Moreover, phenolic contents and antioxidant activities of ethanolic and acetone extracts were investigated.

Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid and butyl-hydroxyanisole (BHA) were purchased from Sigma Chemical (St Louis, MO, USA). All other chemicals were of analytical grade.

Plant material

The aerial parts of three individuals of *M. aegyptiaca* spaced at a distance of about 20 m were collected from south-eastern Tunisia (Medenine: Boughrara area at 19 m altitude, latitude 33°30′91″ N, longitude 10°38′26″ E, with an arid climate characterized by a mean rainfall of 150 mm/year) and identified according to the flora of Tunisia (Le Floc'h and Boulos 2008). For the volatile compound analysis, aerial parts of the plant were collected at the juvenile stage characterized only by young leaves (stage 1; December 2009), at the flowering stage characterized by leafy flowering stems and immature fruits (stage 2; February 2010) (Figure 1) and at mature stage characterized only by mature fruits without any flowers (stage 3; May 2010). After harvest, the fresh vegetable matter was dried on the shadow, until constancy of the weight (20 days), then ground into fine powder and stored at ambient temperature in a dry place and in the dark until use. The physicochemical composition of leaves and mature fruits and the preparation of plant extracts were realized at mature stage (stage 3).

Volatile compounds extraction

The dry matter of leaves, flowers and/or fruits collected during three different stages of *M. aegyptiaca* growth was submitted to hydrodistillation for 4 h, using a Clevenger-type apparatus and the collector solvent used was n-hexane (2 ml). After evaporation of the solvent under nitrogen flow, the volatile extract was dried over anhydrous sodium sulfate and stored in sealed vials protected from the light at −20°C until analysis by GC and GC/MS.

Lipidic component extraction

The lipidic components from leaves and fruits were extracted using chloroform/methanol as previously described by Zouari et al. (2010). After lipid extraction, methyl esters of the fatty acids were

Figure 1. *M. aegyptiaca* L. collected at the flowering stage (stage 2), characterized by leafy flowering stems and immature fruits, from south-eastern Tunisia (Medenine: Boughrara area at 19 m altitude, latitude 33°30′91″ N, longitude 10°38′26″ E).

prepared as follows. A sample containing 50 mg lipids was dissolved in 500 μl n-hexane. Then, 200 μl potassium hydroxide 2 M in methanol was added and the solution was mixed for 2 min in a vortex mixer. After phase separation, the upper layer of n-hexane containing the fatty acid methyl esters was analyzed by GC and GC/MS.

Volatile and lipidic compound analyses

Gas chromatography. The volatiles or lipidic components were analyzed using a Hewlett-Packard 5890 series II gas chromatograph equipped with an HP-5MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm; Hewlett-Packard, Palo Alto, CA, USA) and connected to a flame ionization detector (FID). The column temperature was programmed at 50°C for 1 min, then 7°C/min to 250°C, and then left at 250°C for 5 min. The injection port temperature was 240°C and that of the detector 250°C (split ratio: 1/60). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min, and the analyzed sample volume was 2 μl. Percentages of the constituents were calculated by electronic integration of FID peak areas, without the use of response factor correction. Retention indices (RIs) were calculated for separate compounds relative to C_7–C_{25} n-alkane mixture (Aldrich Library of Chemical Standards, Saint-Louis, Missouri, USA).

Gas chromatography/mass spectrometry. The volatiles or lipidic components were also analyzed by GC/MS, using a Hewlett-Packard 5890 series II gas chromatograph. The fused HP-5MS capillary column (the same as that used in the GC analysis) was coupled to a HP 5972A masse-selective detector (Hewlett-Packard). The oven temperature was programmed at 50°C for 1 min, then 7°C/min to 250°C, and then left at 250°C for 5 min. The injection port temperature was 250°C and that of the detector 280°C (split ratio: 1/100). The carrier gas was helium (99.995% purity) with a flow rate of 1.2 ml/min, and the analyzed sample volume was 2 μl. The mass spectrometer conditions were as follow: ionization voltage, 70 eV; ion source temperature, 150°C; electron ionization mass spectra were acquired over the mass range 50–550 *m/z*.

Volatiles and lipidic components identification. The volatiles or lipidic components were identified by comparing the mass spectra data with spectra available from the Wiley 275 mass spectra libraries (software, D.03.00, Palo Alto, California, USA). Further identification confirmations were made referring to RI data generated from a series of known standards of n-alkane mixture (C_7–C_{25}) and to those previously reported in the literature.

Physicochemical composition and mineral concentrations

Dry matter was determined by oven-drying at 105°C to constant mass (AOAC 1997). Crude proteins were analyzed according to the Kjeldhal method. A factor of6.25 was used for conversion from total nitrogen to crude protein (AOAC 1997). The fat content was determined by Soxhlet extraction with hexane for 8 h at boiling point of the solvent. The ash content was determined by combustion of the sample at 550°C for 8 h. Then, different mineral constituents (potassium [K], calcium [Ca], magnesium [Mg], iron [Fe], sodium [Na], zinc [Zn], manganese [Mn], chromium [Cr] and copper [Cu]) were analyzed separately using an atomic absorption spectrophotometer (Hitachi Z6100, Tokyo, Japan). The carbohydrate content was estimated by difference of mean values:

$$\text{Carbohydrate} = [\text{total solids} - (\text{protein} + \text{lipids} + \text{minerals})].$$

Preparation of M. aegyptiaca *extracts*

The dried powder of the *M. aegyptiaca* aerial part (25 g) was Soxhlet-extracted with 300 ml acetone followed with 300 ml ethanol during 6 h for each solvent. Each solvent was then evaporated using a rotary evaporator and the residual solvent was removed by flushing with nitrogen. Finally, the obtained extracts were kept in the dark at +4°C until further analysis.

Total phenolic content

The total phenolic content of *M. aegyptiaca* extracts was determined by the Folin–Ciocalteu method (Barros et al. 2010). Gallic acid monohydrate was used as standard for the calibration curve. Total phenolic content was expressed as mg gallic acid equivalent (GAE)/g extract.

Antioxidant activity

The DPPH radical-scavenging activity of *M. aegyptiaca* extracts was measured according to the method described by Kirby and Schmidt (1997). The extract concentration providing 50% of radicals scavenging activity (EC_{50}) was calculated from the graph of scavenging activity percentage against the extract concentration. The reducing power of *M. aegyptiaca* extracts was determined by the method of Yildirim et al. (2001) and the extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration.

Statistical analysis

All analytical determinations were performed at least in duplicate. Values were expressed as the mean

Table I. Mean percentage of *M. aegyptiaca* volatile compounds during different phonological stages.

Compound		Concentration (%)[a]			RI[b]
		Stage 1	Stage 2	Stage 3	
1	1-Undecyne	–	1.46	–	1,022
2	*p*-Cymene	–	0.63	–	1,025
3	Benzene acetaldehyde	–	0.34	–	1,046
4	γ-Terpinene	–	3.08	–	1,059
5	α-Terpinolene	–	–	0.12	1,099
6	Nonanal	–	0.6	–	1,102
7	**Camphor**[c]	**5.24**	–	**0.28**	**1,146**
8	Berneol	–	–	0.89	1,168
9	Terpinen-4-ol	–	–	0.24	1,179
10	*α-Terpineol*	–	–	0.17	1,192
11	*trans*-Carveol	–	–	0.33	1,221
12	**Methyl thymyl ether**[c]	–	**10.8**	**0.22**	**1,234**
13	Nonanoic acid	–	–	1.00	1,279
14	Bornyl acetate	–	–	0.94	1,285
15	**4-Vinyl-2-methoxy phenol**[c]	–	**4.58**	–	**1,318**
16	Terpenyl acetate	–	–	3.76	1,348
17	*trans*-β-Damascenone	–	0.61	1.28	1,384
18	β-Ionone	–	1.57	1.28	1,486
19	Bicyclogermacrene	–	0.63	–	1,499
20	β-Sesquiphellandrene	–	0.12	–	1,524
21	Dodecanoic acid	–	–	1.36	1,566
22	Megastigmatrienone	–	–	0.32	1,582
23	Spathulenol	–	–	0.52	1,584
24	Caryophyllene oxide	–	–	3.72	1,590
25	Viridiflorol	–	–	0.89	1,599
26	**Dillapiole**[c]	–	**55.15**	**1.54**	**1,630**
27	β-Eudesmol	–	–	1.16	1,659
28	Pentadecanal	–	0.94	0.55	1,709
29	Tetradecanoic acid	–	–	3.35	1,764
30	**Neophytadiene**[c]	–	**7.95**	**5.15**	**1,829**
31	2-Pentadecanone, 6,10,14-trimethyl	–	1.77	3.24	1,835
32	9-Hexadecenoic acid methyl ester	0.89	–	–	1,890
33	**Hexadecanoic acid methyl ester**[c]	**19.1**	–	**0.45**	**1,909**
34	**Hexadecanoic acid**[c]	**0.83**	–	**20.75**	**1,958**
35	Heptadecanoic acid methyl ester	0.14	–	–	1,994
36	**9,12-Octadecadienoic acid methyl ester**[c]	**20.61**	–	**0.64**	**2,088**
37	9,12,15-Octadecatrienoic acid methyl ester	–	–	1.58	2,097
38	9-Octadecenoic acid methyl ester	1.38	–	–	2,102
39	**Phytol**[c]	–	**4.39**	**11.92**	**2,112**
40	**Octadecanoic acid methyl ester**[c]	**22.65**	–	–	**2,123**
41	9,17-Octadecadienal	1.23	–	–	2,142
42	**Ethyl linoleolate**[c]	–	–	**18.76**	**2,156**
43	Octadecanoic acid	–	–	1.13	2,167
44	**5, 8, 11,14-Eicosatetraenoic acid methyl ester**[c]	**9.26**	–	–	**2,259**
45	7,10,13-Eicosaterienoic acid methyl ester	1.03	–	–	2,277
46	Tricosane	0.35	–	–	2,295
47	11,14-Eicosadienoic acid methyl ester	0.87	–	–	2,296
48	Tetracosane	0.35	–	–	2,394
49	Pentacosane	–	–	0.66	2,500
	Total	83.93	94.62	88.2	

Stage 1, juvenile stage; stage 2, flowering stage; stage 3, mature stage. Compounds are listed in order of their elution from a HP-5MS column. Results are mean values of duplicate injection of three samples. Standard deviations from means did not exceed 1% of absolute values. [a] Percentages obtained by FID peak-area normalization; [b] RIs calculated against C_7–C_{25} n-alkane mixture on the HP 5MS column; [c] Main compound in bold font.

± standard deviation ($n = 3$). Analysis of variance was conducted, and differences between variables were tested for significance by one-way analysis of variance using SPSS 11 (Statistical Package for the Social Sciences, The Predictive Analytics Company, Chicago, IL, USA). A difference was considered statistically significant when $P \leq 0.05$.

Results and discussion

Chemical composition of volatile compounds

The volatile compounds of leaves, flowers and/or fruits collected during three different phonological stages of *M. aegyptiaca* were extracted by hydrodistillation. The yields of volatiles decreased from the juvenile

Table II. Moisture (g/100 g fresh weight) and macronutrient composition (g/100 g dry weight) of *M. aegyptiaca* leaves and fruits.

	Leaves	Fruits
Moisture	74.10 ± 0.60^{A}	72.12 ± 0.40^{B}
Carbohydrates[a]	78.80 ± 1.46^{A}	79.03 ± 1.27^{A}
Proteins	8.70 ± 0.50^{A}	8.0 ± 0.56^{A}
Fat	3.68 ± 0.40^{A}	5.13 ± 0.60^{B}
Ash	8.82 ± 0.56^{A}	7.84 ± 0.11^{B}

Data presented as the mean ± standard deviation ($n = 3$). In each row, different uppercase superscript letters indicate significant differences ($P < 0.05$). [a]The carbohydrate content was calculated by difference: [total solids − (protein + lipids + minerals)].

stage (stage 1) (0.10%, w/w), to the flowering stage (stage 2) (0.06%, w/w) to the mature stage (stage 3) (0.03%, w/w). Similar results were previously obtained for *Artemisia campestris*, where the yields of volatiles decreased from the vegetative stage, full-flowering plants to seed-bearing plants. Nevertheless, the yields of the volatiles from *M. aegyptiaca* were relatively low as compared with those of aromatic plants such as *A. campestris* (Juteau et al. 2002) or *Thymus algeriensis* (Zouari et al. 2011).

The volatile compounds obtained at different stages of the vegetative cycle were analyzed by GC and GC/MS techniques. The components identified at each stage, their percentages and their experimental RIs are listed in Table I. Forty-nine compounds were identified during the three different stages of *M. aegyptiaca* growth (Table I). Fourteen and 16 compounds were identified at the juvenile (stage 1) and at the flowering stages (stage 2), accounting for 83.93% and 94.62% of the total volatile compounds, respectively. Interestingly, many more ($n = 31$) components were identified at the mature stage (stage 3), accounting for 88.20% of the total volatile compounds. The three different stages of *M. aegyptiaca* growth showed a large difference in the qualitative and quantitative composition of volatile compounds. At the juvenile stage (stage 1), the methylated fatty acids (octadecanoic acid methyl ester, 22.65%; 9,12-octadecadienoic acid methyl ester, 20.61%; hexadecanoic acid methyl ester, 19.10%; and 5,8,11,14-eicosatetraenoic acid methyl ester, 9.26%) were the most abundant components, constituting 71.62% of the total volatile compounds.

As can be seen from Table I, at the flowering stage (stage 2) volatiles were characterized by the appearance of a high number of terpenic compounds (monoterpenes, sesquiterpenes and diterpene) at the expense of methylated fatty acids. In fact, dillapiole (55.15%), methyl thymyl ether (10.80%), neophytadiene (7.95%), 4-vinyl-2-methoxy phenol (4.58%) and phytol (4.39%) were the major components accounting for 82.87% of the total volatile compounds (Table I). It was reported that the major component (dillapiole, 55.15%) possessed interesting biological activities (de Almeida et al. 2009). In fact, it was shown that dillapiole presented a fungicide action against the fungus *Clinipellis perniciosa* by inhibition of its spores, in concentrations ranging from 0.6 to 1.0 ppm. Dillapiole was also shown to present larvicide and insecticide actions against the larvae and the adult insects of *Anopheles marajoara* and *Aedes aegypti* (malaria and dengue mosquitoes) (de Almeida et al. 2009).

At the mature stage (stage 3), the main components were hexadecanoic acid (20.75%), ethyl linoleolate (18.76%), phytol (11.92%) and neophytadiene (5.15%), representing 56.58% of the total volatile compounds, followed by terpenyl acetate (3.76%), caryophyllene oxide (3.72%), tetradecanoic acid (3.35%) and 6,10,14-trimethyl-2-pentadecanone (3.24%). It is interesting to point out that there were important quantitative differences as compared with the other stages, suggesting that the maturation of the plant strongly influenced the chemical composition of volatiles. For example, dillapiole and methyl thymyl ether that were found to be the major constituents at the flowering stage were detected only in very low concentrations at the mature stage. In contrast, the concentration of phytol increased from 4.39% (flowering stage) to 11.92% (mature stage).

These major variations of volatile compounds during the various growth stages are probably closely related to the metabolic and physiological changes of the plant during its vegetative cycle. In fact, Schwob et al. (2004) studied the variation of chemical composition of *Hypericum perforatum* essential oil during the phenological cycle and they supposed that, during this physiological process of ontogenesis, the occurring morphological modifications were concomitant with modifications in secondary metabolism. Furthermore, volatiles play an important role in the protection of the plants as antibacterials, antivirals, antifungals, insecticides and also against herbivores by reducing their appetite for such plants. They also may attract some insects to favor the dispersion of pollens and seeds, or repel undesirable others (Bakkali et al. 2008).

Table III. Mineral concentrations (mg/100 g dry weight) in *M. aegyptiaca* leaves and fruits.

	Leaves	Fruits
K	$2{,}111 \pm 55^{A}$	$4{,}130 \pm 80^{B}$
Ca	$1{,}733 \pm 35^{A}$	$1{,}110 \pm 22^{B}$
Mg	554 ± 15^{A}	944 ± 20^{B}
Fe	65.0 ± 0.15^{A}	22.0 ± 0.14^{B}
Na	4.30 ± 0.06^{A}	14.0 ± 0.15^{B}
Zn	<0.05	11.40 ± 0.10
Mn	6.30 ± 0.06^{A}	2.20 ± 0.05^{B}
Cr	8.0 ± 0.10^{A}	2.50 ± 0.05^{B}
Cu	<0.50	2.50 ± 0.04

Data presented as the mean ± standard deviation ($n = 3$). In each row, different uppercase superscript letters indicate significant differences ($P < 0.05$).

Leaf and fruit physicochemical compositions

The results of the nutrient composition (protein, carbohydrate, fat and ash) expressed on a dry weight basis are presented in Table II. Carbohydrates were the most abundant macronutrients for both leaves and fruits (78.8–81 g/100 g), as was found previously for *M. sylvestris* (Barros et al. 2010). The protein content of *M. aegyptiaca* leaves (8.7 g/100 g) was lower than that reported for *M. sylvestris* leaves (12.25 g/100 g). Nevertheless, the protein content of *M. aegyptiaca* fruits (8 g/100 g) was found to be much higher than the value reported for *M. sylvestris* fruits (3.26 g/100 g). However, protein contents of *Malva* species remain much lower than that of other edible leafy green vegetables such as *Spinacea oleracea* (spinach),

Table IV. Mean percentage of lipidic compounds extracted from *M. aegyptiaca* leaves and fruits.

	Compound	Concentration (%)[a]		RI[b]
		Leaves	Fruits	
1	Heptane, 4-methyl	0.11	–	764
2	1-Heptene, 2,4-dimethyl	0.37	–	840
3	Hexanoic acid methyl ester (C6:0)	–	0.27	925
4	1-Undecyne	–	0.62	1,022
5	α-Thujone	0.11	–	1,088
6	5-(1′-1′-dimethylethyl)-Bicyclo [3,1,0] hexan-2-one	1.36	–	1,105
7	**l-Camphor[c]**	**43.69**	–	**1,153**
8	Borneol	0.35	–	1,169
9	1-Dodecene	–	0.11	1,187
10	Nonanoic acid methyl ester (C9:0)	–	0.22	1,220
11	1-Tetradecene	–	0.23	1,386
12	Phenol, 2,4-bis(1,1-dimethylethyl)	0.22	–	1,517
13	6-Tridecene, 7-methyl	0.14	–	1,526
14	1-Nonene, 4,6,8-trimethyl	0.23	–	1,534
15	1-Hexadecene	–	0.18	1,585
16	Tetradecanoic acid methyl ester (C14:0)	0.47	2.31	1,717
17	Neophytadiene	1	–	1,827
18	9-Hexadecenoic acid methyl ester (C16:1)	0.20	0.56	1,889
19	**Hexadecanoic acid methyl ester[c] (C16:0)**	**8.59**	**18.83**	**1,909**
20	Methyl-3-(3,5-ditertbutyl-4-hydroxyphenyl) propionate	0.17	–	1,927
21	Cyclopropane octanoic acid, 2-hexyl-methyl ester	–	0.62	1,973
22	Hexadecanoic acid, 14-methyl-methyl ester	0.18	–	1,989
23	Heptadecanoic acid methyl ester (C17:0)	–	0.31	1,994
24	**9,12-Octadecadienoic acid (Z,Z)-methyl ester[c] (C18:2 n-6)**	**3.49**	**36.17**	**2,088**
25	**9,12,15-Octadecatrienoic acid methyl ester (Z,Z,Z)[c] (C18:3 n-3)**	**14.69**	–	**2,101**
26	**9-Octadecenoic acid methyl ester[c] (C18:1)**	–	**13.85**	**2,102**
27	**Phytol[c]**	**5.32**	–	**2,111**
28	**Octadecanoic acid methyl ester[c] (C18:0)**	**3.80**	**6.63**	**2,123**
29	9,17-Octadecadienal, (Z)	0.95	–	2,140
30	Ethyl linoleate	–	0.62	2,158
31	Methyl-2-octyl cyclopropene-1-octanoate	–	1.77	2,167
32	Cyclopropane octanoic acid-2-octyl-methyl ester	–	0.90	2,206
33	Tricosane	0.38	–	2,294
34	Eicosanoic acid methyl ester **(C20:0)**	0.73	0.45	2,324
35	Tetracosane	0.44	–	2,393
36	Heneicosanoic acid methyl ester **(C21:0)**	0.20	–	2,424
37	1-Eicosanol	0.29	0.13	2,486
38	Pentacosane	0.87	–	2,500
39	Glycerol 2-hexadecanoate	0.92	–	2,511
40	Docosanoic acid methyl ester **(C22:0)**	0.76	0.28	2,523
	Total	90.02	85.06	
	Saturated fatty acids	14.55	29.30	
	Monounsaturated fatty acids	0.2	14.41	
	Polyunsaturated fatty acids	18.18	36.17	
	Oxygenated monoterpenes	45.50	0.0	
	Diterpenes	6.32	0.0	
	Functionalized hydrocarbons (alcohols, aldehydes and esters)	2.34	4.04	
	Hydrocarbons (saturated and unsaturated)	2.54	1.14	
	Phenolics	0.39	0.0	

Lipidic fractions were methylated before analysis by GC and GC/MS. Results are mean values of duplicate injection of three samples. Standard deviations from means did not exceed 1% of absolute values. Compounds are listed in order of their elution from a HP-5MS column. [a] Percentages obtained by FID peak-area normalization; [b] RIs calculated against C_7–C_{25} n-alkane mixture on the HP 5MS column; [c] Main compound in bold font.

Vernonia amygdalina, *Solanum africana*, *Amaranthus hybridus* and *Telfaria occidentalis*, in which protein contents ranged from 30.0 to 34.6 g/100 g dry weight (Aletor et al. 2002, Lisiewska et al. 2011). The ash content varied between 7.84 g/100 g in leaves and 8.82 g/100 g in fruits. Those contents were lower than those reported for *M. sylvestris* leaves (13.53 g/100 g) and fruits (12.83 g/100 g) (Barros et al. 2010).

Concentrations of different minerals (K, Ca, Mg, Fe, Na, Zn, Mn, Cr and Cu) in leaves and fruits are shown in Table III. Mineral contents (Ca, Mg, K and Fe) of *M. aegyptiaca* are comparable with those reported for leaves of various edible wild plants species (Guil et al. 1998) and are much higher than those of several leafy vegetables such as *V. amygdalina*, *S. africana*, *A. hybridus* and *T. occidentalis* (Aletor et al. 2002). The intake of *M. aegyptiaca* could be expected to contribute a large proportion of the essential mineral requirement in the body. In fact, 100 g *M. aegyptiaca* fruits (dry weight) provides more than the recommended daily intake of some minerals (350 mg Mg, 800 mg Ca, 8 mg Zn and 2 μg Cu) per day for an adult (Guil et al. 1998).

Fat was the less abundant macronutrient and its content was found to be comparable with that reported for *M. sylvestris* leaves and fruits (Barros et al. 2010) and for various edible leafy vegetables (Uusiku et al. 2010). Compositions of the lipidic fractions extracted from *M. aegyptiaca* leaves and fruits were investigated using both GC and GC/MS techniques. The percentages and the RIs of the identified lipidic components are listed in Table IV in the order of their elution on the HP-5MS column. The global chromatographic analysis resulted in the identification of 40 compounds, accounting for 90.02% and 85.06% of the total lipidic content of leaves and fruits, respectively. Interestingly, lipidic fraction extracted from *M. aegyptiaca* leaves was characterized by a relatively high rate of phytol (5.32%) and a very high rate of camphor (43.69%) which were not detected in the fruits (Table IV). Interestingly, camphor (oxygenated monoterpene) has been reported to exhibit pronounced antimicrobial activity (Setzer et al. 2004) and was reported to be a major constituent of various antibacterial essential oils. Furthermore, camphor acts as a counter-irritant, rubefacient and mild analgesic, and is included in liniments for relief of fibrositis and neuralgia. By ingestion, camphor has carminative properties and has been used as a mild expectorant and to relieve griping (Royal Pharmaceutical Society of Great Britain 1996).

As compared with leaves, fruits presented the highest contents of polyunsaturated fatty acids (36.17%), saturated fatty acids (29.03%) and monounsaturated fatty acids (14.41%) (Table IV). Besides, the leaves' lipidic fraction contains a relatively high level of linoleinic acid (n-3) (14.69%), which is absent in fruits' lipidic fraction. Fruits' lipidic fraction was characterized by its higher contents of linoleic acid (n-6) (36.17%), oleic acid (13.85%) and palmitic acid (18.83%) as compared with those of leaves. Furthermore, palmitic acid (C16:0) was found to be the main saturated fatty acid found in both samples (leaves and fruits), followed by stearic acid (C18:0). Linolenic acid (n-3), linoleic acid (n-6) and palmitic acid were also found to be the major fatty acids of *M. sylvestris* from north-eastern Portugal (Barros et al. 2010). *M. aegyptiaca* is therefore an important source of n-3 and n-6 polyunsaturated fatty acids that are beneficial to the human health, since their intake would lower the risk of developing artherosclerosis and cholesterol accumulation in the blood (Appel et al. 2005). Furthermore, dietary linolenic acid (n-6) has been assessed for its role in prevention of coronary heart disease (Mozaffarian 2005). It is worth noting that other lipids were identified in both fruits and leaves, such as saturated and unsaturated hydrocarbons ranging from 1.14 to 2.54% of the total lipidic fractions and functionalized hydrocarbons ranging from 2.34 to 4.04% of the total lipidic fractions (Table IV).

Antioxidant potential

Phenolic contents. The yield of extractable compounds relative to the weight of dried plant material ranged from 1.12 g/100 g (ethanol extract) to 2.7 g/100 g (acetone extract) (Table V). It is well known that phenolic substances contribute directly to the antioxidant activity of plant materials. In fact, phenolic compounds exhibit considerable free radical-scavenging activities (through their reactivity as hydrogen-donating or electron-donating agents) and metal ion-chelating properties (Rice-Evans et al. 1996). Therefore, the amounts of total phenols in the extracts were determined (Table V). Our results showed that the content of phenolics varied from 352

Table V. Extraction yields, total phenolic contents and antioxidant potential of ethanol and acetone extracts from *M. aegyptiaca*.

	Acetone extract	Ethanol extract	BHA
Yield (g/100 g dry weight)	2.70 ± 0.20^{A}	1.12 ± 0.10^{B}	–
TPC[a] (mg GAE/g extract)	404 ± 8^{A}	352 ± 6^{B}	–
DPPH scavenging activity[b] (mg/ml)	0.38 ± 0.05^{A}	0.57 ± 0.07^{B}	0.14 ± 0.01
Reducing power[b] (mg/ml)	0.12 ± 0.01^{A}	0.18 ± 0.02^{A}	0.070 ± 0.01

Data presented as the mean ± standard deviation ($n = 3$). In each row, different uppercase superscript letters indicate significant differences ($P < 0.05$). [a] Total phenolic content as GAE; [b] Measured as EC_{50} values.

to 404 mg GAE/g extract. Comparable results were previously obtained for methanol extracts from *M. sylvestris* (Barros et al. 2010).

DPPH radical scavenging activity. Free radical scavenging activities of *M. aegyptiaca* extracts were measured by DPPH assay (Table V). Acetone and ethanol extracts were able to reduce the stable free radical DPPH with EC_{50} values of 0.38 and 0.57 mg/ml, respectively. Our results are comparable with those obtained for various *M. sylvestris* methanol extracts (Barros et al. 2010). The acetone extract, which contained the highest amount of total phenolics, showed a higher free radical-scavenging activity than ethanol extract ($P < 0.05$). These results are in agreement with the fact that free radical-scavenging activity is greatly influenced by the phenolic composition of the sample (Cheung et al. 2003).

Reducing power. The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron, which is an important mechanism of phenolic antioxidant action (Yildirim et al. 2000). Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts (Yildirim et al. 2001). In this assay the ability of *M. aegyptiaca* extracts to reduce Fe^{3+} to Fe^{2+} was determined. Table V also shows the reducing power (as indicated by the extract concentration providing 0.5 of absorbance value) of the different extracts compared with BHA as standard. The acetonic and the ethanolic extracts showed an important reducing power ($EC_{50} = 0.12–0.18$ mg/ml) (Table V). Although BHA presents the highest antioxidant activity, natural antioxidants were of growing interest as compared with synthetic ones.

Conclusion

The present paper is a contribution to the studies of the nutraceutical potential (antioxidant properties), lipid chemical profile, minerals and volatile compounds of leaves and fruits from *M. aegyptiaca*, an edible wild plant largely distributed in North-Africa. In relation to its phenological stages, the studied plant was characterized by an important chemical variability of volatile compounds. Interestingly, *M. aegyptiaca* seems to be a good source of several important nutrients such as essential minerals, n-3 and n-6 fatty acids and other valuable lipidic components known for their interesting biological properties. Furthermore, this vegetable possesses an important antioxidant activity and thus has the potential to be used as a cheap natural source for reducing cellular oxidative damage. In fact, integrating wild vegetables such as *M. aegyptiaca* into diets may be considered a practical and sustainable way to achieve optimal dietary requirements and to combat micronutrient deficiencies. We also think that *M. aegyptiaca* may have other medical and cosmetic applications for sensitive, irritated and dry skin care. Further studies could be undertaken to study the anti-bacterial, soothing and moisturizing properties of extracts of this plant.

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References

Aletor O, Oshodi AA, Ipinmoroti K. 2002. Chemical composition of common leafy vegetables and functional properties of their leaf protein concentrates. Food Chem 78:63–68.

AOAC. 1997. Official Methods of Analysis. 16th ed., Washington, DC: Association of Official Analytical Chemists.

Appel LJ, Sacks FM, Carey VJ. 2005. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids. Results of the OmniHeart Randomized Trial. J Am Med Assoc 294:2455–2464.

Bakkali F, Averbeck S, Averbeck D, Idaomar M. 2008. Biological effects of essential oils—A review. Food Chem Toxicol 46: 446–475.

Barros L, Carvalho AM, Ferreira CFR. 2010. Leaves, flowers, immature fruits and leafy flowered stems of *Malva sylvestris*: A comparative study of the nutraceutical potential and composition. Food Chem Toxicol 48:1466–1472.

Billeter M, Meier B, Sticher O. 1991. 8-Hydroxyflavonoid glucuronides from *Malva sylvestris*. Phytochemistry 30:987–990.

Chaieb M, Boukhriss M. 1998. Flore suscinte et illustrée des zones arides et sahariennes de Tunisie. Sfax, Tunisia: Association de la Protection de la Nature et de l'Environnement, l'Or du Temps. p 103.

Cheung LM, Cheung PCK, Ooi VEC. 2003. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem 81: 249–255.

de Almeida RR, Souto RN, Bastos CN, da Silva MH, Maia JG. 2009. Chemical variation in *Piper aduncum* and biological properties of its dillapiole-rich essential oil. Chem Biodivers 6: 1427–1434.

Espin JC, Garcia-Conesa MT, Tomas-Barberan FA. 2007. Nutraceuticals: Facts and fiction. Phytochemistry 68: 2986–3008.

Guil JL, Gimenez JJ, Torija ME. 1998. Mineral nutrient composition of edible wild plants. J Food Compos Anal 11: 322–328.

Juteau F, Masotti V, Bessiene JM, Viano J. 2002. Compositional characteristics of essential oil of *Artemisia campestris* var. *glutinosa*. Biochem Syst Ecol 30:1065–1070.

Juturu V. 2008. Omega 3 fatty acids and the cardiometabolic syndrome. J Cardiometab Syndr 3:244–253.

Kirby AJ, Schmidt RJ. 1997. The antioxidant activity of Chinese herbs for eczema and of placebo herbs. J. Ethnopharmacol 56: 103–108.

Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF. 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. Am J Med 30:71–88.

Le Floc'h E, Boulos L. 2008. Flore de la Tunisie, Catalogue Synonymique Commenté, Montepellier, France, 461.

Lisiewska Z, Kmiecik W, Gebczynski P, Sobczynska L. 2011. Amino acid profile of raw and as-eaten products of spinach (*Spinacia oleracea* L.). Food Chem 126:460–465.

Mozaffarian D. 2005. Does α-linolenic acid intake reduce the risk of coronary heart disease? A review of the evidence. Altern Ther Health M 11:24–30.

Rice-Evans CA, Miller NJ, Paganga G. 1996. Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20:933–956.

Royal Pharmaceutical Society of Great Britain. 1996. Martindale: The Extra Pharmacopoeia. 31st ed. Reynolds JEF, editor. London: Royal Pharmaceutical Society of Great Britain.

Schwob I, Bessiere JM, Masotti V, Viano J. 2004. Changes in essential oil composition in Saint John's wort (*Hypericum perforatum* L.) aerial parts during its phenological cycle. Biochem Syst Ecol 32:735–745.

Setzer WN, Vogler B, Schmidt JM, Leahy JG, Rives R. 2004. Antimicrobial activity of *Artemisia douglasiana* leaf essential oil. Fitoterapia 75:192–200.

Takeda K, Enoki S, Harborne JB, Eagles J. 1989. Malonated anthocyanins in Malvaceae: Malonylmalvin from *Malva sylvestris*. Phytochemistry 28:499–500.

Uusiku NP, Oelofse A, Duodu KG, Bester MJ, Faber M. 2010. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. J Food Compos Anal 23:499–509.

Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V. 2000. Comparison of antioxidant and antimicrobial activity of tilia (*Tilia argentea* Desf. ex. D.C.), sage (*Salvia triloba* I.) and black tea (*Camellia sinensis*) extracts. J Agric Food Chem 48: 5030–5034.

Yildirim A, Mavi A, Kara AA. 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J Agric Food Chem 49:4083–4089.

Zouari N, Elgharbi F, Fakhfakh N, Ben Bacha A, Gargouri Y, Miled N. 2010. Effect of dietary vitamin E supplementation on lipid and colour stability of chicken thigh meat. Afr J Biotechnol 9: 2276–2283.

Zouari N, Fakhfakh N, Zouari S, Bougatef A, Neffati M, Ayadi MA. 2011. Chemical composition, angiotensin I-converting enzyme inhibitory, antioxidant and antimicrobial activities of essential oil of Tunisian *Thymus algeriensis* Boiss. et Reut. (Lamiaceae). Food Bioprod Process. doi:10.1016/j.fbp.2010.11.006.