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RESEARCH ARTICLE

Antioxidant, anticholinesterase and tyrosinase inhibition activities, and fatty acids of *Crocus mathewii* – A forgotten endemic angiosperm of Turkey

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ABSTRACT

Context We report the first ever chemical/biochemical study on *Crocus mathewii* Kerndorff (Iridaceae) – a Turkish endemic angiosperm. This plant has never been explored for its phytochemistry and bioactivities.

Objective This study explores *C. mathewii* corm and aerial parts for the chemical and biological properties of hexane, ethyl acetate, methanol and water fractions of the extracts.

Material and methods Plant material (20 g) was extracted by methanol (250 mL × 5, 3 days each) and fractioned into hexane, ethyl acetate, methanol and water. All fractions were subjected to β-carotene–linoleic acid, $DPPH^{\cdot}$, $ABTS^{\cdot+}$, CUPRAC, metal chelating and tyrosinase inhibition activities. Hexane fractions were submitted to GC–MS analysis.

Results Ethyl acetate fractions showed excellent IC_{50} values in $DPPH^{\cdot}$ (aerial 36.21 ± 0.76 and corm 33.87 ± 0.02 mg/L) and $ABTS^{\cdot+}$ (aerial 33.01 ± 0.79 and bulb 27.87 ± 0.33 mg/L); higher than the IC_{50} of the standard α-tocopherol (DPPH 116.25 ± 1.97; ABTS 52.64 ± 0.37 mg/L), higher than BHA in DPPH (57.31 ± 0.25 mg/L), but slightly lower in ABTS (19.86 ± 2.73 mg/L). Methanol extract of aerial parts also showed higher activity than α-tocopherol in DPPH (85.56 ± 11.51 mg/L) but slightly less (72.90 ± 3.66 mg/L) than both the standards in ABTS. Linoleic (aerial 53.9%, corm 43.9%) and palmitic (aerial 22.2%, corm 18%) were found as the major fatty acids.

Discussion and conclusion Some fractions of *C. mathewii* showed higher antioxidant activities than the standards. There is a need to explore more about this plant.

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ABTS; β-carotene; CUPRAC; DPPH; GC–MS analysis

Introduction

Crocus belongs to subfamily Crocoideae of Iridaceae, a family of around 80 genera and almost 1500 species (Goldblatt 1990; Bremer et al. 2009). *Crocus* is a genus of 90 flowering species that are grown from the corm (Wani & Mohiddin 2009); most of the species are cultivated for ornamental purposes. *Crocus* species are native to woodland, scrub and meadows. They grow from sea level to high altitudes in Europe, North Africa, Middle East, Aegean and China (Rudall 1994).

Crocus is derived from Greek word 'krokos' that means saffron (*Crocus sativa* L.). *Crocus* species are very important from both a historical and commercial point of view. Harvest and cultivation of *C. sativus* were first documented in the Mediterranean Crete Island (Hogan 2007). Some *Crocus* species are called 'autumn crocus' as they flower in autumn, i.e., September–October.

Crocus mathewii Kerndorff, the plant under study, also known as 'Dream Dancer' (Figure 1), was discovered in 1994 by Helmut Kerndorff and Erich Pasche (Kerndorff 1988) in south Turkey. Its distribution is restricted to a few regions of Taurus Mountain. It grows during long summer days at dry mountain slopes from 400 to 1100 m altitudes. It can be characterized by its corm and whitish flowers with a dark purple zone in the throat (Figure 1). *C. mathewii* was named in the honour of Brian Mathew who was famous as the '*Crocus* guru' (Grey-Wilson & Mathew 1981). He proposed the properties for the taxonomic classification of *Crocus* (Petersen et al. 2008). There was confusion regarding how to differentiate between *C. mathewii* and *C. asumaniae* (Mathew & Beytop 1976). However, taxonomists consider them different species due to their different habitats.

C. mathewii is an infant plant to the scientific community. A careful literature survey does not provide any phytochemical investigation or biological activities of this plant. One can expect biological importance of *C. mathewii* after studying its vastly studied sister species. For example, the water:methanol (50:50, v/v)

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Figure 1. Image of *C. mathewii* at the time of collection.

extract of *C. sativus* have been reported to possess better antioxidant activities; even higher than that of tomatoes and carrots (Papandreou et al. 2006). On the contrary, Ramadan et al. (2012) has reported weak antioxidant activity of the ethanol extract of the same plant, although they concluded the extract as non-toxic. Sariri et al. (2011) studied the antioxidant and antityrosinase activities of extract obtained from the methanol extract of *C. sativus* flowers. They reported the extract as 30% antityrosinase inhibitor, although they stated the extract as antioxidant on the basis of total phenolics present in the extract. Karimi et al. (2010) studied free radical scavenging and ferric reducing activities of the *C. sativus* stigma and found notable activities, i.e., 68.2% and 78.9%, at a concentration of 300 µg/mL. This plant has also been reported to have antibacterial (Parray et al. 2014) and inhibitory activity on amyloid-β-aggregation (Papandreou et al. 2006).

Based on the medicinal properties of neighbour species, we decided to explore scientific windows on *C. mathewii*. Herein, we report the first scientific work performed on this species that includes the antioxidant, anticholinesterase and tyrosinase inhibitory activities as well as the GC–MS analysis.

Materials and methods

Crocus mathewii was collected from Fethiye-Babadag Mountains in Mugla Province of Turkey during October 2014. The plant was characterized by Kenan Akba (MSc) and Ömer Varol (PhD); working at the herbarium of the Biology Department of Mugla University. A specimen (voucher number K.A 625) was submitted at the stated herbarium. About 20 g of the plant was dried by wrapping it in a filter paper for 60 days – unexposed to light.

Extraction and fractionation

Plant material was divided into two parts: corm and aerial parts including flowers. Each part was crushed into small pieces (approx. 10 g of each) and transferred into 250 mL methanol. Extracts were obtained after 3 days and this process was repeated until the solvent became colourless. The crude extract was evaporated to dryness and dissolved in distilled water. The aqueous solution was fractionated into hexane, ethyl acetate, methanol and water.

Determination of antioxidant activity

All antioxidant activities were performed according to the standard literature procedures with slight modifications (Öztürk et al. 2011). Total antioxidant activity was evaluated using β-carotene–linoleic acid test (Marco 1968). Free radical scavenging activity was determined spectrophotometrically by the DPPH radical assay (Blois 1958). The spectrophotometric analysis of $ABTS^{\cdot+}$ scavenging activity was determined according to the literature method (Re et al. 1999). Superoxide anion radical scavenging activity was performed according to Liu's method (Liu et al. 1997). CUPRAC antioxidant activity was performed according to Apak's procedure (Apak et al. 2004). The extract was also tested for metal chelating activity on Fe^{2+} spectrophotometrically (Decker & Welch 1990). α-Tocopherol and BHA were used as standards in β-carotene–linoleic acid, $DPPH^{\cdot}$, $ABTS^{\cdot+}$, and CUPRAC assays. EDTA was used as a standard in metal chelating assay.

Determination of anticholinesterase activity

Acetylcholinesterase and butyrylcholinesterase inhibitory activities were measured by the spectrophotometric method developed by Ellman et al. (1961).

Tyrosinase activities

Tyrosinase enzyme inhibitory activity was measured according to Khatib at al. (2005) procedure with slightly

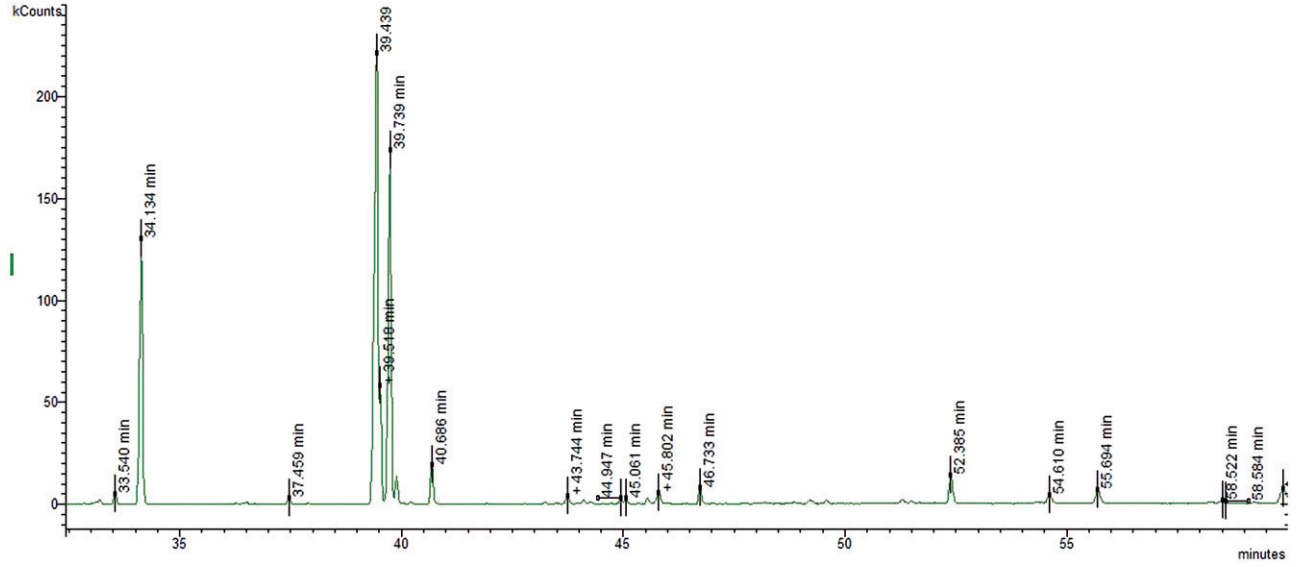

Figure 2. Chromatogram of hexane fraction from the corm of *C. mathewii*.

modified Hearing (1987) spectroscopic method. Mushroom tyrosinase enzyme was used with L-DOPA as a substrate, while kojic acid was used as a standard tyrosinase inhibitor. Kojic acid was used as a standard.

Fatty acid compositions

Esterification of acids

Crocus mathewii (20 g) crude material was dried, grind to powder and extracted with 250 mL of hexane (6 × 24 h) at room temperature. Crude hexane extract (100 mg) was vacuum filtered and dissolved in 2 mL of 0.5 mol L^{-1} sodium hydroxide solution. This mixture was heated on a water bath (below 50 °C). Two millilitres of BF_3–MeOH complex was introduced to the reaction mixture and boiled for 3 min. After cooling, the mixture was diluted with saturated sodium chloride solution (25 mL). The synthesized esters were extracted with *n*-hexane, washed with potassium bicarbonate solution (4 mL, 2%) and dried over anhydrous Na_2SO_4. Solvent was evaporated to obtain methyl esters (Gören et al. 2006).

Gas chromatography

GC parameters were set according to Topcu et al. (2013). Fatty acids were analyzed according to the procedure of Tel et al. (2013). GC chromatograms of the hexane fractions, obtained from corm and aerial parts of *C. mathewii*, are given in Figures 2 and 3, respectively. Gas chromatography–mass spectrometry

All instrumental parameters to determine fatty acids methyl esters in the samples were set according to the Tel et al. (2013).

Determination of IC_{50} values

The results obtained from biological activities are expressed as 50% inhibition concentration (IC_{50}). The sample concentration, which provides 50% biological activity (IC_{50}), was calculated from the graph of % activity versus sample concentration.

Statistical analysis

Antioxidant and tyrosinase inhibitory activity data (Table 1) are the averages of triplicate analyses. Data were recorded as mean ± standard error of mean (SEM). Significant differences between means were determined by Student's *t*-test, while p values <0.05 were regarded as significant.

Results and discussion

Hexane, ethyl acetate, methanol and water fractions were obtained from both corm and aerial parts of *C. mathewii*. Separately, 5 g of plant material (combined corm and aerial parts) was boiled in water for 30 min to obtain water boiled fraction; that was used to prepare silver nanoparticles in a separate study. After nanoparticle separation through centrifuge, the remaining fraction was termed as SM1 that was extracted with hexane that yielded another nonpolar fraction SM2.

As a whole, 10 obtained extracts were subjected to antioxidant activities, i.e., β-carotene–linoleic acid, $DPPH^{\cdot}$, $ABTS^{\cdot+}$, CUPRAC and metal chelating activities (Table 1).

Ethyl acetate fraction of corm and aerial parts showed excellent IC_{50} values in DPPH and ABTS assays; even the

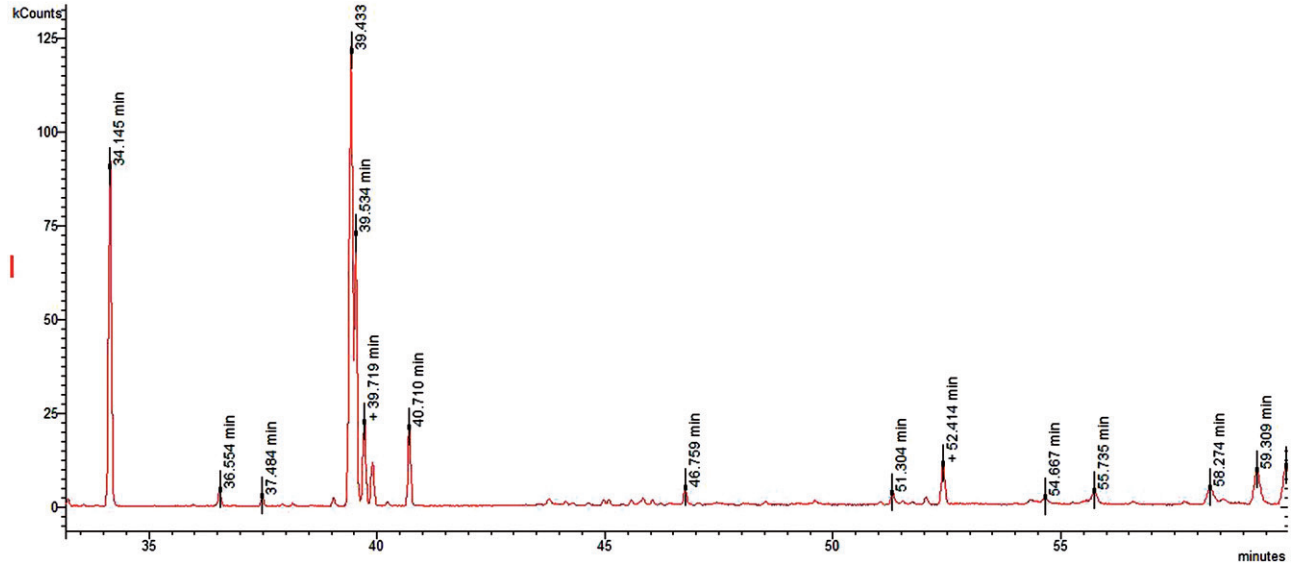

Figure 3. Chromatogram of hexane fraction from the aerial parts of *C. mathewii*.

Table 1. β-Carotene–linoleic acid, DPPH$^{\cdot}$, ABTS$^{\cdot+}$, CUPRAC and metal chelating antioxidant, and tyrosinase activities of the extracts obtained from *Crocus mathewii*.[a]

Samples	β-Carotene–linoleic acid IC_{50} (mg/L)	DPPH$^{\cdot}$ assay IC_{50} (mg/L)	ABTS$^{\cdot+}$ assay IC_{50} (mg/L)	Metal chelating assay IC_{50} (mg/L)	CUPRAC assay IC_{50} (mg/L)	Tyrosinase IC_{50} (mg/L)
A-Hexane	8.69 ± 0.13	8311.00 ± 1483.51	1429.34 ± 212.12	315.85 ± 9.87	296.50 ± 10.61	1658.69 ± 26.30
B-Hexane	14.47 ± 0.65	1003.68 ± 1.12	1525.14 ± 5.96	10 004.82 ± 385.73	280.67 ± 29.02	2239.37 ± 44.08
A-EtOC	51.88 ± 2.2	36.21 ± 0.76	33.01 ± 0.79	591.71 ± 142.10	47.15 ± 2.09	NA
B-EtOC	15.63 ± 0.2	33.87 ± 0.02	27.87 ± 0.33	NA	46.70 ± 2.97	NA
A-MeOH	59.10 ± 0.22	85.56 ± 11.51	72.90 ± 3.66	NA	145.50 ± 2.12	NA
B-MeOH	76.99 ± 11.23	314.33 ± 6.33	320.99 ± 5.47	na	422.33 ± 5.86	NA
A-H_2O	211.17 ± 19.55	266.38 ± 6.19	439.81 ± 34.70	947.61 ± 120.14	338.67 ± 35.12	NA
B-H_2O	393.79 ± 1.38	801.02 ± 34.55	1217.90 ± 347.09	947.77 ± 98.10	1001.33 ± 103.38	NA
SM1	2.26 ± 0.05	NA	200.11 ± 16.37	168.20 ± 11.33	344.33 ± 18.72	NA
SM2	NA	NA	990.76 ± 122.76	107.05 ± 8.89	653.53 ± 52.78	NA
α-Tocopherol[b]	2.10 ± 0.08	116.25 ± 1.97	52.64 ± 0.37		0.85 ± 0.02	
BHA[b]	1.34 ± 0.04	57.31 ± 0.25	19.86 ± 2.73		2.42 ± 0.01	
EDTA[b]				3.47 ± 0.35		
Kojic acid						14.07 ± 1.68

NA, not active; BHA, butylatedhydroxyl anisole; EDTA, ethylenediaminetetraacetic acid.
[a]IC_{50} values represent the means + SEM of three parallel measurements ($p < 0.05$).
[b]Reference compounds.

values were higher than the standards α-tocopherol and BHA. In DPPH, the aerial ethyl acetate fraction showed IC_{50} value of 36.21 ± 0.76 mg/L, while the corm reflected 33.87 ± 0.02 mg/L. This bioactivity is higher than both α-tocopherol (IC_{50} 116.25 ± 1.97 mg/L) and BHA (IC_{50} 57.31 ± 0.25 mg/L). Assimopoulou et al. found higher DPPH radical scavenging activities in the methanol extract of *C. sativus* and assigned the activity to crocin – a component found in the stated plant (Assimopoulou et al. 2005). Chen et al. (2008) has also found higher DPPH activities in ethanol extract of *C. sativus* and found 107 mg/g of α-tocopherol equivalent. However, Karimi et al. (2010) has claimed that *C. sativus* collected from Khorasan province of Iran reflected higher DPPH activities; even higher than reported by Assimopoulou et al. (2005) and Chen et al. (2008).

In ABTS assay of ethyl acetate fraction, aerial parts produced IC_{50} value of 33.01 ± 0.79 mg/L, while the corm provided 27.87 ± 0.33 mg/L. These values were higher than α-tocopherol (52.64 ± 0.37 mg/L) but slightly lower than BHA, i.e., 19.86 ± 2.73 mg/L (Table 1). Serrano-Díaz et al. (2012) also observed ABTS scavenging activities in *C. sativus* when they were studying the antioxidant activities of flowers and stigmas. Keyhani et al. (2007) studied the corm of *C. sativus* for several days and found better ABTS activity when the root elongated for 6 days.

Among the all tested fractions, both corm and aerial ethyl acetate fractions showed the highest CUPRAC activity (IC_{50} mg/L: aerial 47.15 ± 2.09, corm 46.70 ± 2.97), though the values were less than the standard α-tocopherol (IC_{50} 0.85 ± 0.02 mg/L) and EDTA (Table 1). In metal chelating assay, ethyl acetate

fraction of corm did not show any bioactivity, while the aerial part fraction showed lower activity (IC_{50} 591.71 ± 142.10 mg/L) than EDTA (IC_{50} 3.47 ± 0.35 mg/L). Sariri et al. (2011) used ascorbic acid to measure reducing power of *C. sativus* extract and found 193.91 ± 1.22 of IC_{50} (ascorbic acid IC_{50}: 15.69 ± 0.08) that was a bit higher than our *C. mathewii*.

Methanol extract of *C. mathewii* also showed quite noticeable antioxidant activities. The aerial parts showed higher activity than α-tocopherol in the DPPH assay (IC_{50} 85.56 ± 11.51 mg/L), while it also reflected ABTS assay value very close to the standards (IC_{50} 72.90 ± 3.66 mg/L). On the other hand, this fraction did not show any metal chelating assay activity, the aerial part fraction showed bioactivity that should interest the reader (IC_{50} 145.50 ± 2.12 mg/L) (Table 1).

As a whole, hexane, water and methanol fraction of corm showed lower antioxidant activities. It is also quite interesting that the boiled fractions (SM1 and SM2) showed the highest metal chelating activity as compared to the other fractions (Table 2). SM2 showed higher metal chelating activity (IC_{50} 107.05 ± 8.89 mg/L) as compared to SM1 (IC_{50} 168.20 ± 11.33 mg/L).

Hexane, ethyl acetate, methanol and water extracts obtained from corm and aerial parts were subjected to acetyl- and butylcholinesterase activities. All the extracts showed very less activity against both the enzymes.

Tyrosinase is a multifunctional copper-containing oxidase enzyme that catalyses the first two steps in mammalian melanogenesis. It initiates the enzymatic browning reactions in harvested fruits. Hyperpigmentation is undesirable in human skin similar to the enzymatic browning of fruits. Researchers are in a quest to seek new potent tyrosinase inhibitors that could be safely used in foods and cosmetics (Chang 2009).

A kaempferol isolated from *C. sativus* has been reported to have tyrosinase inhibitory activity (ID_{50} 67 µg/mL, 0.23 mM) (Kubo & Kinst-Hori 1999). In this study, hexane extracts of both corm and aerial parts of *C. mathewii* showed slight inhibition of tyrosinase activities (Table 1). Aerial parts showed slight higher inhibition (IC_{50} 1658.69 ± 26.30 mg/L) as compared to the hexane fraction of the corm (IC_{50} 2239.37 ± 44.08 mg/L). However, both activities were lower than the standard kojic acid (IC_{50} 14.07 ± 1.68 mg/L). The remaining fractions did not show any noticeable inhibition of tyrosinase activity. Sariri et al. (2011) found tyrosinase inhibitory activity with an IC_{50} value 9132.55 ± 278.72 (ascorbic acid IC_{50}: 229.68 ± 1.06) in *C. sativus* that is less than the value observed in *C. mathewii* in the current study.

GC–MS analysis of the aerial and corm of *C. mathewii* showed 18 fatty acids; among these, 13 were observed in the aerial parts while 16 in the corm extract (Table 2). There are previous reports on the sister species of *C. mathewii* that shows more or less similar fatty acids. Yayli et al. (2001) reported 22 fatty acids from *Crocus vallicola*. Both aerial parts and corm of *C. mathewii* showed less total saturation (39.6% and 26.8%, respectively) as compared to the total unsaturation (60.4% and 73.2%). In contrast, *C. vallicola* showed more total saturation than unsaturation (Yayli et al. 2001). Saturation/unsaturation ratio in aerial and corm extracts was found as 0.6 and 0.4, respectively. Linoleic acid/oleic acid ratio was found as 9.6 and 1.8 in aerial parts and corm, respectively. Among the fatty acids, *Z*-linoleic acid was observed as the most abundant in both aerial (35.7%) and corm (38.8%) extracts of *C. mathewii*.

Table 2. The fatty acid composition (%) of aerial part and corm of *Crocus mathewii*.

Fatty acid	Aerial part (%)	Corm (%)
Z-9-palmitoleic acid (C16:1, Δ^9)	–	0.6
Palmitic acid (C16:0)	22.2	18.0
Hexadecanoic acid. 9-Methly (C17:0)	0.9	–
Margaric acid (C17:0)	0.6	0.4
Z,Z-Linoleic acid ($C_{18:2}$, $\Delta^{9.12}$)	**35.7**	**38.8**
E,E-Linoleic acid (C18:2)	16.2	5.1
Oleic acid ($C_{18:1}$, Δ^9)	5.6	25.0
Elaidic acid (C18:1)	2.9	1.3
Stearic acid (C18:0)	5.4	2.5
Arachidonic acid (20:4)	–	0.4
Gondoic acid (C20:1)	–	0.9
Arachidic acid (C20:0)	1.2	1.1
Behenic acid (C22:0)	3.4	2.3
Tricosenoic acid (C23:1)	–	0.7
Tricosanoic acid (C23:0)	1.1	1.5
Tetracosenoic acid (C24:1)	–	0.4
Tetracosanoic acid (C24:0)	4.8	1.0
Total saturation	39.6	26.8
Total unsaturation	60.4	73.2
Saturation/unsaturation	0.6	0.4
L/O[a]	9.6	1.8

L/O[a], linoleic acid–oleic acid ratio.
Bold values show the highest amount of fatty acids obtained from aerial and corm parts.

A previous study on *Crocus pelistericus* showed palmitic (57.4%) and linolenic (42.6%) acids as the major fatty acids (Jankuloski et al. 2014). On the contrary, we found 53.9% linoleic acid (*Z*-linoleic 35.7%, *E*-linoleic 16.2%) in aerial parts while 43.9% in corm (*Z*-linoleic 38.8%, *E*-linolenic acid 5.1%). In addition, 22.2% and 18.0% palmitic acid was observed in the aerial and corm of *C. mathewii*, respectively.

Other fatty acids observed in *C. mathewii* are *Z*-9-palmitoleic acid (C16:1, Δ^9), 9-methlyhexadecanoic acid (C17:0), margaric acid (C17:0), oleic acid ($C_{18:1}$, Δ^9), elaidic acid (C18:1), steraic acid (C18:0), elaidic acid (C18:1), stearic acid (C18:0), arachidonic acid (20:4), gondoic acid (C20:1), arachidic acid (C20:0), behenic acid (C22:0), tricosenoic acid (C23:1), tricosanoic acid

(C23:0), tetracosenoic acid (C24:1), tetracosanoic acid (C24:0) (Table 2).

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Declaration of interest

The authors declare no conflicts of interest.

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