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Occurrence of secondary metabolites in tepals of *Asphodelus ramosus* L.

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Abstract
Major processes contributing to subtleties of ephemeral flowers of *Asphodelus ramosus* are related to chemical constituents detected in tepals which expand during cold and wet seasons in the eastern Mediterranean. Luteolin, caffeic acid, chlorogenic, and *p*-hydroxy-benzoic acids are the main constituents, whereas alkanes, ketones, and fatty acids appear in low amounts.

Keywords: Asphodelus ramosus, *flower, secondary metabolites, tepals*

Introduction

Asphodelus ramosus L. (Asphodelaceae) is a perennial geophyte, consistent floristic element of the Mediterranean ecosystems and the most common species of the genus *Asphodelus* with branched inflorescences (Narducci 1957; Díaz Lifante & Valdés 1994; Días Lifante & Aguinagalde 1996; Días Lifante & Valdés 1996). The flowering period of *A. ramosus* starts in February and ends in March, coinciding with the rainy and cold seasons in the eastern Mediterranean. The ephemeral, actinomorphic flowers of *A. ramosus* open acropetally by exhibiting six whitish tepals on flowering stalks that may be up to 1 m long (Argiropoulos 2009).

This study was conducted in an effort to identify secondary metabolites related to adaptations of delicate tepals of *A. ramosus* exposed to unfavorable, abiotic, environmental conditions in the eastern Mediterranean. In the subterranean tissues of other *Asphodelus* species (i.e., *A. albus* Mill., *A. acaulis* Desf., *A. fistulosus* L., and *A. microcarpus* Viv.), lipophilic anthranoid aglycones (chrysophanol, asphodeline, and O,7′-bichrysophanol), β-sitosterol-β-D-glucoside, and aryl coumarin glycoside (asphodelin A 4′-*O*-β-D-glucoside) have been identified (Hammouda et al. 1972; Van Wyk et al. 1995; El-Seedi 2007). Also, alkaloids have been investigated in the leaves of *A. aestivus* Brot. and *A. tenuifolius* Cav. (Çalış et al. 2006; Vagashiya et al. 2011). In contrast, very little information has been obtained regarding the secondary metabolites of flowers, which contribute to defensive functions (Müller & Riederer 2005), whereas a phytochemical analysis of tepals of *A. ramosus* has not hitherto been reported.

Materials and methods

Plant material

Flowers of *A. ramosus* were randomly collected early in March, from natively growing plants in the Campus of the University of Athens (UoA) in Greece (250 m a.s.l., 37°57.6′N, 23°47.1′E), where monthly precipitation was estimated at 50 mm and average temperature was 14°C. The plant material was identified by Assistant Professor Dr Theophanis Constantinidis (Department of Biology, Section of Systematics & Ecology, University of Athens, Greece). A voucher specimen (UoA CR-14) has been deposited at the Herbarium of the University of Athens, Greece.

Phytochemical analysis

Dried tepals of flowers were ground (19.6 g) and extracted with a mixture of MeOH–H_2O (5:1) (3 × 240 ml) under agitation in a shaker for 90 min, the

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combined extracts were filtered and concentrated to dryness in vacuum. The obtained residue (14.0 g) was prefractionated by vacuum liquid chromatography (VLC) over silica gel (5.5 cm × 10 cm) using eluents mixtures of cycloxexane–CH_2Cl_2–MeOH–H_2O (100:0:0:0, 0:95:5:0–0:20:80:0, 0:0:50:50) of increasing polarity to yield 11 fractions (A–K) of 300 ml. Fraction A (6.3 mg, eluted with cyclohexane) was analyzed through Gas Chromatography-Mass Spectrometry (GC-MS). Fractions F and G were combined (300 mg eluted with CH_2Cl_2–MeOH, 80:20 and 70:30, respectively) and were further purified by column chromatography (CC) over Sephadex LH 20 eluted with CH_2Cl_2–MeOH (20:80) and yielded luteolin (205.4 mg). Fraction I (4.5 g eluted with CH_2Cl_2–MeOH, 20:80) was further purified by CC over Sephadex LH 20 (MeOH) and afforded nine fractions (I_1–I_9). Fraction I_5 was identified as chlorogenic acid (4.5 mg). Fraction I_2 (373.9 mg) was further purified by CC over silica gel using as eluents mixtures of CH_2Cl_2–MeOH–H_2O (from 95:5:0.1 to 70:30:3) and yielded seven fractions (IB_1–IB_7). Fraction IB_6 was identified as *p*-hydroxy-benzoic acid (3.2 mg). Fraction I_4 (62.4 mg) was further purified by High Performance Liquid Chromatography (HPLC) (AcOH 5%–MeOH, 65:35) and yielded 11 fractions (IC_1–IC_{11}). Fraction IC_4 was identified as caffeic acid (11.4 mg). ^1H, ^{13}C, and 2D NMR spectra were recorded in CD_3OD on Bruker DRX 400 and Bruker AC 200 (50.3 MHz for ^{13}C NMR) instruments at 295 K. Chemical shifts are given in ppm (δ) and were referenced to the solvent signals at 3.31 ppm and 49.5 ppm for ^1H and ^{13}C NMR, respectively. Correlation Spectroscopy, Heteronuclear Single Quantum Coherence, and Heteronuclear Multiple Bond Correlation were carried out using standard Bruker microprograms. Infra Red spectra were obtained on a Perkin-Elmer PARAGON 500 FT-IR spectrophotometer. Ultraviolet-Visible spectra were recorded on a Shimadzu UV-160A spectrophotometer, according to Mabry et al. (1970). Optical rotations were measured on a Perkin-Elmer 341 polarimeter. VLC was carried out on silica gel 60H (Merck Art. 7736). CC was carried out on silica gel (Merck Art. 9385), gradient elution with the solvent mixtures indicated in each case; Sephadex LH-20 (Pharmacia). Thin Layer Chromatography: Merck siliga gel 60 F_{254} (Art. 5554). Detection: UV–light, spray reagent [vanillin–H_2SO_4 on silica gel]; Merck, Cellulose (Art. 5552); Neu's reagent. Preparative HPLC was carried out on Jasco system MD-2015 Plus Multiwavelength Detector equipped with a PU 2089 pump Plus Quaternary Gradient Pump (Jasco Corporation, Tokyo, Japan), using a reversed phase column, Kromasil C_{18} Semi-prep (250 mm × 10 mm). Analysis was carried out on a Hewlett-Packard 6890 gas chromatograph fitted with a fused silica HP-5 MS capillary column that was temperature programed from 50°C to 280°C at a rate of 4°C min^{-1}. Helium was used as carrier gas at a flow rate of 0.8 ml min^{-1}. The chromatograph was coupled to a MS 5973 mass selective detector at 70 eV. The spectra were taken in electron impact mode. Retention indices for all compounds were determined according to the van den Dool approach using *n*-alkanes as standards. The identification of the compounds was based on comparison of their mass spectra with those of Wiley and NBS Libraries (Massada 1976) and those described by Adams (2006), as well as on comparison of their retention indices with literature values.

Results

Chromatographic separation of the polar extract of tepals of *A. ramosus* yielded luteolin (0.5 mg g^{-1} d.w.), caffeic acid (0.6 mg g^{-1} d.w.), chlorogenic acid (0.22 mg g^{-1} d.w.) and *p*-hydroxy-benzoic acid (0.15 mg g.$^{-1}$ d.w.). Results from GC–MS analysis of the nonpolar constituents eluted with cyclohexane (fraction A) are given in Table I. Notably, several hydrocarbons (54.6%) were identified in homologous series, i.e., alkanes (C_{12}–C_{29}: 51.6%), alkene (C_{20}: 3%), and related aldehydes (C_9–C_{24}: 18.1%). In addition, two ketones and two fatty acids were present in appreciably low amounts (0.4% and 0.5%, respectively). Concerning the identified hydrocarbons, docosane and tricosane were the most abundant (Table I).

Discussion

Thirty-eight secondary metabolites were detected in tepals of ephemeral flowers of *A. ramosus* that blossom during the cold and rainy seasons in Mediterranean region (Table I). Among them luteolin, which is present in leaves of *A. ramosus* and floral tissues of other species, can enhance protection against UV-B radiation, herbivores, and microorganisms (Reynaud et al. 1997; Çalış et al. 2006; Ohno et al. 2011). Caffeic acid is a component related to herbivores, which can form esters in the side chain of acylated anthocyanins (Matsuoka et al. 2011). Chlorogenic acid serves as a pigment and antioxidant in ornamental plants (Olszewska & Kwapisz 2011). *p*-Hydroxy-benzoic acid acts as antibacterial and antifungal, and it is involved in flowers' pigmentation (Pandey et al. 2011). Hence, secondary metabolites contribute to flowers' coloration that is associated with protective and defensive mechanisms of floral tissues, against herbivores and microbes (Guzmán et al. 2011).

Components of secondary metabolites are typical constituents of the cuticle (Riederer & Müller 2006),

Table I. Nonpolar compounds in the tepals of *Asphodelus ramosus*.

	Compound	R.I.[a]	Peak area (%)
1	Nonanal	1096	0.5
2	Undecanal	1296	0.2
3	*n*-Tetradecane	1400	0.1
4	Dodecanal	1394	0.1
5	Pentadecane	1500	0.1
6	Tridecanal	1489	0.2
7	Hexadecane	1600	0.2
8	Tetradecanal	1589	0.2
9	Heptadecane	1700	0.1
10	Pentadecanal	1710	0.5
11	2-Hexadecanone	1797	0.1
12	Octadecane	1800	0.5
13	Hexadecanal	1813	0.5
14	*n*-Nonadecane	1900	0.2
15	Heptadecanal	1915	0.6
16	Hexadecanoic acid	1973	0.1
17	3-Eicosene	1989	3.0
18	Eicosane	2000	0.9
19	2-Octadecanone	1999	0.3
20	Octadecanal	2016	1.0
21	Heneicosane	2100	1.9
22	Nonadecanal	2122	3.2
23	Z-9-octadecenoic acid	2133	0.4
24	Docosane	2200	11.0
25	Tricosane	2300	11.9
26	Heinecosanal	2315	4.8
27	Tetracosane	2400	8.6
28	Tricosanal	2518	3.1
29	Pentacosane	2500	7.2
30	Tetracosanal	2616	3.2
31	Hexacosane	2600	3.3
32	Heptacosane	2700	2.0
33	Octacosane	2800	2.4
34	Nonacosane	2900	1.2
			73.6

[a] Retention Indexes on HP-5 MS capillary column.

which in the case of *A. ramosus* possesses dense striations on the tepals' surfaces (Argiropoulos 2009). Similarly, wavy cuticular striations were observed on petals of other native species blossoming during the same period in the eastern Mediterranean (Argiropoulos & Rhizopoulou 2012). Cuticular structures on petal surfaces influence the wettability and adhesion properties of floral tissues and form microsculptural features that efficiently mediate interactions between flowers and pollinators (Rands et al. 2011; Argiropoulos & Rhizopoulou 2013).

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References

Adams PR. 2006. Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, IL: Allured Publishing Corporation.

Argiropoulos A. 2009. On the colour of flowers. PhD thesis. University of Athens, Greece, 223 pp.

Argiropoulos A, Rhizopoulou S. 2012. Topography and nanosculpture of petals' surfaces of short-lived flowers of the wild species *Cistus creticus, Cistus salviifolius*, *Eruca sativa* and *Sinapis arvensis*. Bot Stud 53: 479–488.

Argiropoulos A, Rhizopoulou S. 2013. Morphological features of petals of *Nerium oleander* L. Plant Biosyst, doi:10.1080/11263504.2013.763863.

Çalış I, Birincioğlu SS, Kırmızıbekmez H, Pfeiffer B, Heilmann J. 2006. Secondary metabolites from *Asphodelus aestivus*. Z Naturforsch 61b: 1304–1310.

Días Lifante Z, Aguinagalde I. 1996. The use of random amplified polymorphic DNA (RAPD) markers for the study of taxonomical relationships among species of *Asphodelus* Sect. *Verinea* (Asphodelaceae). Am J Bot 83: 949–953.

Díaz Lifante Z, Valdés B. 1994. Lectotypification of *Asphodelus ramosus* (Asphodelaceae), a misunderstood Linnean name. Taxon 43: 247–251.

Días Lifante Z, Valdés B. 1996. Revisión del género *Asphodelus* L. (Asphodelaceae) en el Mediterráneo Occidental. Genève: Conservatoire et Jardin Botaniques de Genève.

El-Seedi HR. 2007. Antimicrobial arylcoumarins from *Asphodelus microcarpus*. J Nat Prod 70: 118–120.

Guzmán B, Narbona E, Vargas P. 2011. Similar reproductive success of the two petal colour polymorphisms of *Cistus ladanifer* (Cistaceae). Plant Biosyst 145: 931–937.

Hammouda FM, Rizk AM, Ghaleb H, Abdel-Gawad MM. 1972. Chemical and pharmacological studies of *Asphodelus microcarpus*. Planta Med 22: 188–195.

Mabry TG, Markham KR, Thomas MB. 1970. The systematic identification of flavonoids. Berlin: Springer-Verlag.

Massada Y. 1976. Analysis of essential oil by gas chromatography and spectrometry. New York: John Wiley & Sons.

Matsuoka N, Ikeda T, El-Aasr M, Manabe H, Murakami Y, Deguchi H, et al. 2011. Study of the chemical constituents of Pruni Cortex and its related parts. J Nat Med 65: 166–171.

Müller C, Riederer M. 2005. Plant surface properties in chemical ecology. J Chem Ecol 31: 2621–2651.

Narducci A. 1957. Osservazioni sulla morfologia, anatomia e ciclo di sviluppo di *Asphodelus ramosus* L. var. *aestivus*. Brot. Plant Biosyst 64: 319–346.

Ohno S, Hosokawa M, Kojima M, Kitamura Y, Hoshino A, Tatsuzawa F, et al. 2011. Simultaneous post-transcriptional gene silencing of two different chalcone synthase genes resulting in pure white flowers in the octoploid dahlia. Planta 234: 945–958.

Olszewska MA, Kwapisz A. 2011. Metabolite profiling and antioxidant activity of *Prunus padus* L. flowers and leaves. Nat Prod Res 25: 1115–1131.

Pandey DP, Rather MA, Nautiyal DP, Bachheti RK. 2011. Phytochemical analysis of *Abutilon indicum*. Int J Chem Tech Res 3: 642–645.

Rands SA, Glover BJ, Whitney HM. 2011. Floral epidermal structure and flowers orientation: Getting to grips with awkward flowers. Arthropod Plant Interact 5: 279–285.

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Reynaud J, Filament MM, Lussignol M, Becchi M. 1997. Flavonoid content of *Asphodelus ramosus* (Liliaceae). Can J Bot 75: 2105–2107.

Riederer M, Müller C. 2006. Biology of the plant cuticle. Oxford: Blackwell Publishing Ltd.

Vagashiya Y, Dave R, Chanda S. 2011. Phytochemical analysis of some medicinal plants from western region of India. Res J Med Plant 5: 567–576.

Van Wyk B-E, Yenesew A, Dagne E. 1995. Chemotaxonomic significance of anthraquinones in the roots of Asphodeloideae (Asphodelaceae). Biochem Syst Ecol 23: 277–281.

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