A chemosystematic study of *Asphodelus aestivus* Brot. (Asphodelaceae) in Egypt

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Background and objectives
In the Egyptian flora, the genus *Asphodelus* was formally placed in Liliaceae family and recently is ranked as a separate family; Asphodelaceae. *Asphodelus aestivus* Brot. is the one of the five *Asphodelus* spp. distributed wild in Egypt; it grows on rocky or sandy ground and in dry grasslands. Therefore, this study aims to investigate the chemical constituents of *A. aestivus* compared with those reported in the species of families Liliaceae s.l. and Asphodelaceae to evaluate their chemosystematic relationship.
Materials and methods
The chemical components of the defatted hydroalcoholic extract of *A. aestivus* were analyzed using different chromatographic and spectral techniques.
Results and conclusion
A total of 13 flavonoid compounds were isolated from *A. aestivus*. They are identified as kaempferol (1), kaempferol 7-*O*-β-glucopyranoside (2), kaempferol 3-*O*-(6′-α-rhamnopyranosyl)-β-glucopyranoside-7-*O*-α-rhamnopyranoside (3), apigenin (4), apigenin 7-*O*-β-glucopyranoside (5), luteolin (6), luteolin 7-*O*-β-glucopyranoside (7), vitexin (8), isovitexin (9), apigenin 6, 8 di-*C*-β-glucopyranoside (10), saponarin (11), orientin (12), and isoorientin (13). Except for compounds 9 and 13, all compounds were isolated for the first time from *A. aestivus*.
A. aestivus is characterized by flavones, flavonols, and flavone *C*-glycosides. It is thus different from other species of Liliaceae *s.l*. which are characterized by either flavones and flavone *C*-glycosides or flavonols and flavone *C*-glycosides, supporting the placement of the genus *Asphodelus* in a distinct family (Asphodelaceae).

Keywords:
Asphodelaceae, *Asphodelus aestivus*, chemosystematics, flavonoids, Liliaceae *s.l*

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Introduction

The genus *Asphodelus* is recently included in the subfamily Asphodeloideae of the family Asphodelaceae [1,2]. The family comprises 40 genera and 900 species found in the temperate regions and subtropical Africa [3]. In the flora of Egypt, the family is represented by two genera and five species, included within Liliaceae *s.l* [4,5], then in a separate family; Asphodelaceae [1,2]. Asphodelaceae is characterized by the flowers being gamopetalous with six stamens and slightly succulent leaves, usually arranged in rosettes with superior ovary. Economically, the roots of some *Asphodelus* species provide gums, glue, and dye and are used in the fermentation of alcohol [6]. They are also used as antispasmodic, diuretic, and as emmenagogue [7].

The flavones *C*-glycosides; isovitexin, isoorientin, and isoorientin 4′-*O*-β-glucopyranoside, as well as two acylated isoorientin derivatives; 6′′-*O*-(malonyl)-isoorientin and 6′′-*O*-[(S)-3-hydroxy-3-methylglutaroyl]-isoorientin were previously isolated from Asphodelus *aestivus* [8]. Flavones and flavones *C*-glycosides were found in *Asphodelus ramosus* subspp. *ramosus* [9]. Chrysoeriol and luteolin were also isolated from *Asphodelus fistulosus* [10]. The present study aims to investigate the chemical constituents of *A. aestivus* compared with those reported for the species of families Liliaceae *s.l.* and Asphodelaceae to evaluate their chemosystematic relationship.

Materials and methods

Plant material

Fresh aerial parts of *A. aestivus* were collected on the Burg Al Arab road, Egypt in 29 March 2007. The sample was identified by Professor Dr S.A. Kawashty, Department of Phytochemistry and Plant Systematics, National Research Centre. A voucher specimen

(s. n. MS10) has been deposited in the herbarium of the National Research Centre (CAIRC).

Extraction and isolation

The dried aerial parts of *A. aestivus* were ground. The ground material was extracted by repeated percolation with 70% methanol till exhaustion, followed by repeated percolation with 50% methanol; the combined extracts being filtered and the solvent was removed under reduced pressure at 60°C. The crude extracts were defatted with petroleum ether (40–60°C). The extract was fractionated using CC Polyamide S6 (Riedel-de Haën AG, Seelze, Germany), starting with water as eluent, then decreasing the polarity by increasing the concentration of methanol to yield 56 fractions. Similar fractions were combined according to their PC properties (Whatman 3MM, Whatman Ltd., Maidstone, Kent, England) using H_2O, 15% AcOH and BAW as eluents to give four main fractions (A–D). Fraction A was applied to a polyamide column using MeOH : H_2O (1 : 1). Fractionation gave rise to three compounds (3, 10, and 11), which were further purified on PPC using BAW as eluents. Fraction B was subjected to chromatographic elution on PPC using BAW followed by CAW yielded compounds 8, 9, 12, and 13. A fraction C was applied to PPC using BAW as eluent to yieldcompounds 2, 5, and 7. Compounds 1, 4, and 6 were obtained from fractions D through PPC using 15% AcOH and BAW as eluents. Finally, all isolates were purified on Sephadex LH-20 (Pharmazia) with 100% methanol as eluent [11,12].

Kaempferol (1) [13]: yellow amorphous powder, *Rf*=0.74 (BAW). Ultraviolet (UV) spectral data, λ_{max}(nm): (MeOH) 266, 292sh, 319sh, 366; (+NaOMe), 276, 320sh, 411; (+AlCl3) 269, 305, 350, 423; (+$AlCl_3$/HCl) 267, 305, 350, 424; (+NaOAc) 274, 306, 378; (+NaOAc/H_3BO_3) 265, 294, 319, 369.^{1}H nuclear magnetic resonance (NMR) (500 MHz, DMSO-*d6*, *δ*, ppm, J/Hz): 8.03 (2H, d, J=8.5, H-2′, H-6′); 6.93 (2H, d, J=8.5, H-3′, H-5′); 6.41 (1H, d, J=2.0, H-8); 6.17(1H, d, J=2.0, H-6). EIMS; *m/z* 286.1

Kaempferol 7–*O*–β–glucopyranoside (2) [11]: yellow amorphous powders, *Rf*: 0.70 (BAW). UV spectral data, λ_{max}(nm): (MeOH) 267, 360; (+NaOMe) 274, 450; (+$AlCl_3$) 278, 310, 360, 425; (+$AlCl_3$/HCl) 278, 309, 360, 425; (+NaOAc) 276, 360, 400; (+NaOAc/H_3BO_3) 268, 294, 359. ^{1}H-NMR (500 MHz, DMSO-*d6*, *δ*, ppm, J/Hz): 7.97 (2H, d, J=8.5, H-2', H-6'); 6.89 (2H, d, J=8.5, H-3', H-5'); 6.68 (1H, d, J=2.0, H-8); 6.42 (1H, d, J=2.0, H-6); 5.02 (1H, d, J=7.2, H-1′′); 3.0–4.0 (5H, m, overlapped with –OH signals, H-2′′- H-6′′).

Kaempferol 3-*O*-(6′-α-rhamnopyranosyl)-β-glucopyranoside-7-*O*-α-rhamnopyranoside (3) [14]: a pale yellow powder, *Rf*: 0.18 (BAW). UV spectral data, λ_{max}(nm): (MeOH) 266, 353; (+NaOMe) 267, 402; (+$AlCl_3$) 275, 300, 352, 401; (+$AlCl_3$/HCl) 274, 301, 350, 399; (+NaOAc) 265, 355, 40; (+NaOAc/H_3BO_3) 265, 355. ^{1}H-NMR (500 MHz, DMSO-*d*$_6$, *δ*, ppm, J/Hz): 8.1 (2H, d, J=8.5Hz, H-2', H-6'), 6.89 (2H, d, J=8.5 Hz, H-3', H-5'), 6.8 (1H, d, J=2.0 Hz, H-8), 6.45 (IH, d, J=2.0 Hz, H-6), 5.55 (1H, d, J=2.0 Hz, H-1′′), 5.35 (1H, d, J=7.5 Hz, H-1′), 4.4 (1H, d, J=2.0Hz, H-1′′), 3.2-3.9 (9H, hidden under water signals, H-2′→H-6′, H-2′′→H-5′′, and H-2′′′-H-5′′), 1.14 (1H, d, J=6.0 Hz, H-6′′), 1.12 (1H, d, J=6.0 Hz, H-6′′). ^{13}C NMR (125 MHz, DMSO-*d6*, ppm): 178.2 (C-4), 162.2 (C-7), 160.7 (C-4′), 161.4 (C-5), 157.6 (C-9), 156.6 (C-2), 134.3 (C-3), 131.6 (C-2′), 131.6 (C-6′), 121.2 (C-1′), 115.7 (C-3′), 115.7 (C-5′), 106.2 (C-10), 102.0 (C-1′), 100.6 (C-1′′), 99.9 (C-6), 98.9 (C-1′′), 95.2 (C-8), 74.2 (C-3′′), 73.5 (C-5′′), 72.5 (C-2′′), 72.2 (C-4′′), 71.6 (C-4′), 71.1 (C-3′′), 70.9 (C-3′), 70.8 (C-2′′), 70.6 (C-2′), 70.4 (C-4′′), 68.5 (C-5′′), 68.8 (C-5′), 65.8 (C-6′), 18.4 (C-6′′), 18.4 (C-6′′). ESIMS; $(M-H)^-$, *m/z* 739.

Apigenin (4) [13,15]: yellow amorphous powder, *Rf*: 0.72 (BAW). UV spectral data, λ_{max} (nm): MeOH: 272, 331; NaOMe: 268, 332, 400; $AlCl_3$: 279, 306, 351, 387; $AlCl_3$/HCl: 279, 304, 346, 386; NaOAc: 281, 306, 387; NaOAc/H_3BO_3: 268, 342. ^{1}H-NMR (400 MHz, DMSO-*d6*, *δ*, ppm, J/Hz): *δ*=7.92 (2H, d, J=8.5 Hz, H-2′, H-6′), 6.91 (2H, d, J=8.5 Hz, H-3′, H-5′), 6.75 (1H, s, H-3), 6.44 (1H, d, J=2.5 Hz, H-8), 6.14 (1H, d, J=2.5 Hz, H-6). EIMS (*m/z*): 270.2.

Apigenin 7-*O*-*β*-glucopyranoside (5) [13,15]: yellow powder, Rf: 0.51 (BAW). UV spectral data, λ_{max} (nm): MeOH: 273, 332; MeOH/NaOMe: 268, 388; $AlCl_3$: 275, 301, 345, 385; $AlCl_3$/HCl: 275, 300, 340, 395; NaOAc: 267, 347, 389; NaOAc/H_3BO_3: 268, 295, 331. ^{1}H-NMR (500 MHz in DMSO-*d*$_6$, *δ*, ppm, J/Hz): 7.82 (2H, d, J=8.7 Hz, H-2′,H-6′), 6.85 (2H, d, J=8.7 Hz, H-3′, H-5′), 6.75 (1H, s, H-3), 6.72 (1H, d, J=2.0 Hz, H-8), 6.36 (1H, d, J=2.0 Hz, H-6), 5.39 (1H, d, J=7.7 Hz H-1′′), 3.0-4.0 (5H, m, H-2′′–H-6′′).

Luteolin (6) [13]: yellow amorphous powder, *Rf*: 0.66 (BAW). UV spectral data, λ_{max} (nm): MeOH: 255sh, 266, 344; MeOH/NaOMe: 273, 320sh, 408; $AlCl_3$: 271, 299, 360, 398; $AlCl_3$/HCl: 271, 254, 387; NaOAc: 272, 342, 400; NaOAc/H_3BO_3: 259, 370.

^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, J/Hz): δ=7.25 (1H, dd, J=2.0, 8.5 Hz, H-6′),7.22 (1H, d, J=2.0 Hz, H-2′), 6.54 (1H, d, J=8.5 Hz, H-5′), 6.37 (1H, s, H-3), 6.15 (1H, d, J=2.0 Hz, H-8), 5.91 (1H, d, J=2.0 Hz, H-6). EIMS (*m/z*): 286.

Luteolin 7-*O*-β-glucopyranoside (7) [13,15]: yellow powder, *Rf*: 0.34 (BAW). UV spectral data, λ_{max} (nm): MeOH: 255, 267, 348; MeOH/NaOMe: 267, 401; $AlCl_3$: 273, 295, 350, 398; $AlCl_3$/HCl: 264, 292, 349, 387; NaOAc: 266, 349, 405; NaOAc/H_3BO_3: 259, 371. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, *J*/Hz): 7.35 (1H, dd, J=2.0, 8.5 Hz, H-6′),7.27 (1H, d, J=2.0 Hz, H-2′), 6.72 (1H, d, J=2.0 Hz, H-8), 6.62 (1H, d, J=8.5 Hz, H-5′), 6.57 (1H, s, H-3), 6.33 (1H, d, J=2.0 Hz, H-6), 5.02 (1H, d, J=7.7 Hz, H-1′′), 3.0-4.0 (5H, m, H-2′′–H-6′′).

Vitexin (8) [15]: yellow powder, *Rf*: 0.38 BAW. UV λ_{max}; (MeOH) 270, 331; (+NaOMe) 279, 329sh, 394; (+$AlCl_3$) 276, 303, 346, 382; (+$AlCl_3$/HCl) 277, 303, 343, 381; (+NaOAc) 279, 389; (+NaOAc/H_3BO_3) 270, 319, 345. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, J/Hz): δ=7.94 (2H, d, J=8.7 Hz; H-2', H-6'), 6.86 (2H, d, J=8.7 Hz; H-3', H-5'), δ=6.59 (1H, s; H-3), 5.99 (1H, d, J=2.0 Hz; H-6), 4.69 (1H, d, J=9.3 Hz; H-1′), 3.0–4.0 (5H, m, H-2′′–H-6′′).

Isovitexin (9) [15]: yellow powder, *Rf*: 0.52 BAW. UV λ_{max}; (MeOH) 270, 332; (+NaOMe) 279, 330sh, 398; (+$AlCl_3$) 276, 305, 347, 387; (+$AlCl_3$/HCl) 277, 303, 346, 385; (+NaOAc) 279, 389; (+NaOAc/H_3BO_3) 273, 319, 348. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, J/Hz): δ=8.02 (2H, d, J=8.7 Hz; H-2', H-6'), 6.8 (2H, d, J=8.7 Hz; H-3', H-5'), δ=6.75 (1H, s; H-3), 6.46 (1H, s, H-8), 4.6 (1H, d, J=9.3 Hz; H-1′), 3.0–4.0 (5H, m, H-2′′–H-6′′).

Apigenin 6, 8 di-*C*-β-glucopyranoside (10) [13]: yellow amorphous powder, *Rf*: 0.33 (BAW). UV, spectral data, λ_{max} (nm): MeOH: 274, 329; MeOH/NaOMe: 286, 327, 396; $AlCl_3$: 278, 305, 349; $AlCl_3$/HCl: 278, 302, 343; NaOAc: 282, 385; NaOAc/H_3BO_3: 277, 318, 358. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, J/Hz): 7.99 (2H, d, J=8.8 Hz, H-2′, H-6′), 6.86 (2H, d, J=8.8 Hz, H-3', H-5′), 6.78 (1H, s, H-3), 6.5 (1H, s, H-8), 4.72 (1H, d, J=9.6 Hz, H-1′′′), 4.67 (1H, d, J=9.6 Hz, H-1′′), 3.0–4.0 (10H, m, H-2′′–H-6′′, H-2′′′–H-6′′′).

Apigenin 6-*C*-β-glucopyranoside-7-*O*-β-glucopyranoside (saponarin) (11) [13,15]: yellow powder, *Rf*: 0.34 (BAW). UV spectral data, λ_{max} (nm): MeOH: 273, 334; MeOH/NaOMe: 283, 332, 398; $AlCl_3$: 28, 306, 351; $AlCl_3$/HCl: 279, 303, 342, 383; NaOAc: 281, 373; NaOAc/H_3BO_3: 275, 324, 337. ^{1}H-NMR (400 MHz in DMSO-d_6, δ, ppm, J/Hz): δ=8.08 (2H, d, J=8.7 Hz, H-2′, H-6′), 6.94 (2H, d, J=8.7 Hz, H-3', H-5′), 6.77 (1H, s, H-3), 6.75 (1H, s, H-8), 5.02 (1H, d, J=7.2 Hz, H-1′′′), 4.75 (1H, d, J=9.0 Hz, H-1′′), 3.0–4.0 (10H, m, H-2′′- H-6′′, H-2′′′–H-6′′′).

Orientin (12) [15]: yellow powder, *Rf*: 0.36 BAW. UV λ_{max}; (MeOH) 258, 270sh, 350; (+NaOMe) 268, 335, 407; ($AlCl_3$) 275, 300sh, 423; (+$AlCl_3$/HCl) 263, 278sh, 299sh, 362, 389; (+NaOAc) 273, 325sh, 379; (+NaOAc/H_3BO_3) 263, 377, 440. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, J/Hz): δ=7.45 (2H, m; H-2', H-6'), 7.18 (2H, d, J=8.5 Hz; H-5'), δ=6.75 (1H, s; H-3), 6.13 (1H, s; H-6), 4.82 (1H, d, J=10.5 Hz; H-1′), 3.0–4.0 (5H, m, H-2′′–H-6′′).

Isoorientin (13) [16]: yellow powder, *Rf*: 0.44 BAW. UV λ_{max}; (MeOH) 258, 270sh, 350; (+NaOMe) 268, 335, 407; (+$AlCl_3$) 275, 300, 423; (+$AlCl_3$/HCl) 263, 278sh, 299sh, 362, 390; (+NaOAc) 273, 325sh, 379; (+NaOAc/H_3BO_3) 277, 440. ^{1}H-NMR (500 MHz in DMSO-d_6, δ, ppm, J/Hz): δ=7.37 (2H, m; H-2', H-6'), 6.81 (2H, d, J=8.5 Hz; H-5'), δ=6.61 (1H, s; H-3), 6.38 (1H, s; H-8), 4.52 (1H, d, J=10.5 Hz; H-1′), 3.0–4.0 (5H, m, H-2′′–H-6′′).

Results and discussion

Identification of the isolated compounds

The present study dealt with the isolation and characterization of 13 flavonoids from *A. aestivus*. They were identified as kaempferol (1), kaempferol 7-*O*-β-glucopyranoside (2), kaempferol 3-*O*-(6′-α-rhamnopyranosyl)-β-glucopyranoside-7-*O*-α-rhamnopyranoside (3), apigenin (4), apigenin 7-*O*-β-glucopyranoside (5), luteolin (6), luteolin 7-*O*-β-glucopyranoside (7), vitexin (8), isovitexin (9), apigenin 6, 8 di-*C*-β-glucopyranoside (10), saponarin (11), orientin (12), and isoorientin (13). Their structure elucidation was carried out through chemical investigations (mild and complete acid hydrolysis) and physical investigations [UV, Mass (MS), and NMR]. Further confirmation was carried out through comparison of their spectroscopic data with previously reported values [11–17].

Chemotaxonomic significance

Asphodelaceae are formerly placed in Liliaceae *s.l.* either as tribe *Asparagineae* or as subfamily *Asparagoideae* [18–21]. According to the Angiosperm Phylogengy Group (APG) III system [22,23],

Asphodelaceae was not recognized, and instead was treated as subfamily *Hemerocallidoideae* in the expanded family Xanthorrhoeaceae *s.l.* However, Takhtajan [23] treated Asphodelaceae as a distinct family which comprises two subfamilies. On the other hand, Asphodelaceae are treated as belonging to Hemerocallidaceae which comprises three subfamilies for example, Hemerocallidoideae, Xanthorrhoideae, and Asphodeloideae corresponding to the previously described families [24,25]. The most recent APG classification (APG IV) [26] places the three families (Asphodelaceae, Hemerocallidaceae, and Xanthorrhoeaceae) into a single family: Asphodelaceae *s.l.*, these families were previously treated as three subfamilies (Asphodeloideae, Hemerocallidoideae, and Xanthorrhoideae) according to APG III [22,27].

In Egypt, Täckholm [4,28], El Hadidi and Fayed [5] recorded the genus *Asphodelus* together with 19 genera within Liliaceae. Boulos [1,2] distributed these genera into five families, namely, Colchicaceae, Liliaceae, Asphodelaceae (including *Asphodelus*), Hyacinthaceae, and Asparagaceae.

To the best of our knowledge, kaempferol, kaempferol 7-*O*-β-glucopyranoside, kaempferol 3-*O*-(6′-α-rhamnopyranosyl)-β-glucopyranoside-7-*O*-α-rhamnopyranoside, apigenin (4), apigenin 7-*O*-β-glucopyranoside (5), luteolin (6), luteolin 7-*O*-β-glucopyranoside (7), vitexin (8), apigenin 6, 8 di-*C*-β-glucopyranoside (10), saponarin (11), and orientin (12) were isolated for the first time from *A. aestivus*. From the flavonoids point of view, Asphodelaceae is characterized by the presence of flavones and their *O*-glycosides, flavonols and their *O*-glycosides as well as flavone *C*-glycosides. Other families that also previously belonged to the family Liliaceae are characterized by either flavones derivatives and flavone *C*-glycosides or flavonol derivatives and flavone *C*-glycosides [29,30]. Asphodelaceae is not only chemically different from the other Liliaceae *s.l.*, but also morphologically. It is characterized by gamopetalous flowers with six stamens and superior ovaries and rosette succulent or reduced leaves [30].

Conclusion

The flavonoid profile of *A. aestivus* is different from that found in the genera of the Liliaceae, a plant family to which *Asphodelus* used to belong. The present data support the recent nesting of the genus *Asphodelus* to a different family, Asphodelaceae, which does not even belong to the same plant order as the Liliaceae. Further investigation is needed to include the different species of Liliaceae *s.l.* in Egypt for supporting the most resent taxonomic/floristic classification.

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Conflicts of interest

There are no conflicts of interest.

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