Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Chemical Analysis and Cytotoxic Evaluation of *Asphodelus aestivus* Brot. Flowers

Mai M. Farid [a], Maha A. Salem [a]*, Rasha R. Abd El-Latif [a], Ahmed Elkhateeb [a], El-Sayed S. Abdel-Hameed[b], Mona M. Marzouk [a], Sameh R. Hussein[a]

[a]*Department of Phytochemistry and Plant Systematics, National Research Centre, 33 El Bohouth St., Dokki, Giza, Egypt, P. O. 12622.*

[b]*Laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute, Giza, Egypt.*

Abstract

Asphodelus species are among many plants used in traditional medicine, they are used by native peoples for various pathologies. *Asphodelus aestivus* Brot. is one of *Asphodelus* species which is considered an important wild medicinal plant growing mainly at Mediterranean region. The present study aims to investigate the chemical constituents and cytotoxic evaluation of *A. aestivus* flowers. Petroleum ether extract was analyzed by GC/MS assay which revealed the characterization of twenty nine compounds, representing 93.13% of total extract. Also, the chromatographic investigation of the hydromethanolic extract led to the isolation of ten phenolics. They were identified as kaempferol (**1**), kaempferol 3-*O*-glucoside (**2**), kaempferol 3,7-di-*O*-glucoside (**3**), luteolin 7-*O*-glucoside (**4**), isoorientin (**5**), isoorientin 7-*O*-glucoside (lutonarin) (**6**), caffeic acid (**7**) emodin (**8**), apigenin 7-*O*-glucoside (**9**) and isovitexin-7-*O*-glucoside (saponarin) (**10**), from which, five compounds ((**2**, **3**, **6**-**8**) have been isolated for the first time from *A. aestivus*. Furthermore, LC-ESI-MS chemical profiling managed to detect the minor contents and led to the annotation of thirteen additional metabolites. In addition, the petroleum ether and the hydromethanolic extracts were investigated against four human carcinoma cell lines; HepG2, HCT-116, MCF-7 and A549 at concentration of 100 mg/mL. The results showed moderate % of inhibition against MCF-7 (63.8 and 68.4) followed by HepG2 (52.3 and 65.7), respectively. Both extracts showed weak inhibition (5.7-14.3%) on HCT-116 and A549. To the best of our knowledge, this is considered the first report of the chemical analysis and biological evaluation of *A. aestivus* flowers.

Keywords: *A. aestivus*; LC-ESI-MS; GC-MS analysis; Biological activity.

1. Introduction

The genus *Asphodelus* L. (Family Asphodelaceae Juss.) is characterized by its antioxidant and anti-inflammatory properties; it is used as a prophylactic agent against tumors and has inhibitory activity against tyrosine kinase in the melanin production as well [1]. *Asphodelus aestivus* Brot. is one of the five *Asphodelus* species distributed in Egypt [2]. It is a wild geophyte plant grows in dry grass lands and on rocky or sandy soil with basal leaves and short vertical rhizome. Its flowers are pinkish-white, with six perianth segments and about 14–19 mm long in addition to six stamens of the same length, in two whorls. They are actinomorphic, and have a smell similar to the cat pee [1, 2]. This plant is not consumed by wild animals either because of the scent of its flowers or its chemical composition [1]. *A. aestivus* contains several compounds such as glycosides, alkaloids, anthraquinones, flavonoids, anthranoids, and triterpenes [3, 4]. Furthermore, the flower was characterized by its essential oil content [1]. In addition, the plant is described as least concern species on international union for the conservation of nature (IUCN) Red List of Threatened species [5].

*Corresponding author e-mail: maha_abdelmejed@yahoo.com; (Maha Abdelmejed Salem).
Receive Date: 30 March 2021, Revise Date: 21 April 2021, Accept Date: 04 May 2021
DOI: 10.21608/EJCHEM.2021.70226.3546

Economically, the dried tuber is crushed and mixed with cold water to make strong glue [6] that is used by bookmakers and shoemakers [7-9]. A yellow dye is obtained from the tuber [7, 10]. *A. aestivus* has been used in traditional (folk) medicine, the tubers and roots are used against hemorrhoids, nephritis, wounds and burns [11-12]. Roots also were reported to have gastroprotective effect against ethanol-induced lesions [13]. Previous reports of *A. aestivus* leaves showed a powerful antioxidant activity; their antioxidant capacities seem to be related to their chemical compositions [14]. Its active extracts could be used as additives in food, cosmetic and pharmaceutical industries. However, the plant flowers have not been examined before for its biological activity. So, our aim in this study is to investigate the chemical constituents and cytotoxic activity of *A. aestivus* flowers against human hepatic carcinoma (HepG2), Human colon carcinoma (HCT-116), Human breast carcinoma (MCF-7) and Human lung carcinoma (A549) cell lines.

2. Experimental

2.1. Plant material

Flowers of *A. aestivus* were collected from the Burg Al Arab road, Egypt in 28 February 2019. The sample was identified by Professor Dr. S.A. Kawashty, Department of Phytochemistry and Plant Systematics, National Research Centre. A voucher specimen (s.n.MS18) has been deposited in the herbarium of the National Research Centre (code: CAIRC).

2.2. Extraction and isolation

Fifty grams of the air-dried powdered of *A. aestivus* flowers were exhaustively extracted several times with petroleum ether (40-60°C). The solvent was evaporated under reduced pressure at 40ºC. The remaining plant powder (500 g) was extracted three times using 70% MeOH then evaporated under reduced pressure at 70ºC [15]. The petroleum ether extract was subjected to GC-MS analysis to study the volatile constituents. The hydro methanol extract (35 g) was subjected to chromatographic investigation for the separation and identification of pure compounds. They included preparative paper chromatography; PPC (Whatmann 3MM; BAW, H_2O and/or 15% HOAc) and column chromatography (CC) (Sephadex LH-20; 60×2.5 cm; MeOH: H_2O). The isolated compounds were purified on CC (Sephadex LH-20; 35 ×1.5 cm; MeOH). Ten pure compounds were obtained and their structures were elucidated on the basis of chromatographic analysis (R_f values, colour reactions and Co-PC with reference samples); chemical investigation (acid hydrolysis) and physical investigations (UV, NMR, and ESI–MS). Further confirmation was performed by comparing their spectral data with previously reported values. The 70% MeOH extract was further analyzed using LC–ESI–MS analysis to detect the compounds that could not be isolated due to their minority.

2.3. Analysis of petroleum ether extract (GC/MS analysis)

GC/MS analysis of the petroleum ether extract of *A. aestivus* was performed by the method described by Farid et al., [16] using Thermo Scientific capillary gas chromatography (model Trace GC ULTRA) directly coupled to ISQ Single Quadruple MS. The identification of the compounds was carried out by comparison of their retention times and fragmentation patterns of mass with those of published data Adams [17] and/or with those of the Wiley spectral library collection and NIST library.

2.4. Analysis of hydro methanol extract

2.4.1. Acid hydrolysis and paper chromatography

The aqueous methanol extract of *A. aestivus* flowers (100 mg) was hydrolyzed with 10 mL HCl 2N (100°C, 2h). The acidic solution was fractionated with 10 mL ethyl acetate after cooling for several times till exhaustion. The collective ethyl acetate fractions were filtered over anhydrous Na_2SO_4 then evaporated. The ethyl acetate residue was subjected to one dimension paper chromatography (PC) Whatman No. 1 (Whatman Ltd. Maidstone, Kent, England) using solvent systems; 50% AcOH (H_2O-AcOH, 1:1) and BAW (*n*-BuOH-HOAc-H_2O, 4:1:5, upper layer) to detect the aglycones. Also, the aqueous layer was carefully neutralized, then subjected to PC investigation using BBPW (Benzene-*n*-BuOH-pyridine-H_2O, 1:5:3:3, upper layer) to detect the sugars [14]. Flavonoid aglycones, phenolic acids (Fluka AG, Bucns SG, Switzerland) and sugar samples (E. Merck, Darmstadt, Germany) were used as authentic references.

2.4.2. LC-ESI-MS analysis

The analysis system consists of HPLC (Waters Alliance 2695) and mass spectrometry (Waters 3100). The mobile phases were prepared daily by filtering through 0.45 μm membrane disc filter and degassed by sonication before use. The mobile phase for gradient elution consists of two solvents: solvent A

[0.1% formic acid (FA) in H_2O] and solvent B [0.1% FA in CH_3CN/MeOH (1:1; v/v)]. The linear gradient profile was as follows: 95% A (5 min), 95-90% A (10 min), 90-50% A (55 min), 50-95% A (65 min), and 95% A (70 min). The injection volume was 10 µL. The flow rate (0.6 ml/min) was split 1:1 before the MS interface. The negative ion mode parameters were as follows: source temperature 150 °C, desolvation temperature 350 °C, cone gas flow 50 L/h, cone voltage 50 eV, capillary voltage 3 kV, and desolvation gas flow 600 L/h. Spectra were recorded in the ESI negative mode between 50 and 1000 *m/z*. The peaks and spectra were processed using the Masslynx 4.1 software [18]. The compounds isolated in the present study were used as authentic samples in LC-ESI-MS analysis. Known peaks were identified by comparing their retention time and mass spectrum with the standard flavonoids (95% purity; UV, ESI, NMR) isolated in the present study and others which obtained from our research group (phytochemical and plant systematic department, NRC) [19-24]. Other peaks were tentatively identified by comparing their fragmentation pattern with literatures.

2.5. Cytotoxic activity

2.5.1. Cell lines

Human hepatic carcinoma (HepG2), Human colon carcinoma (HCT-116), Human breast carcinoma (MCF-7) and Human lung carcinoma (A549) cell lines were obtained from Vacsera (Giza, Egypt). Culture was maintained in RPMI medium and supplemented with 10% fetal bovine serum at 37 °C in 5% CO_2 and 95% humidity. Cells were sub-cultured using trypsin versene 0.15%.

2.5.2. Cell viability assay

After 24 h of seedling cells in 96 well plates, the medium was changed to serum-free medium containing a final concentration of the extracts of 100µg/ml in triplicates. The cells were treated for 24 h. 0.5% DMSO was used as negative control. Cell viability was determined using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide) assay [25]. The equation used for calculation of percentage cytotoxicity = (1-(av(*x*)/(av(NC))* 100.

Where Av: average, X: absorbance of sample well measured at 595 nm with reference 690 nm, NC: absorbance of negative control measured at 595 nm with reference 690.

3. Results and discussion

3.1. Phytochemical investigation

3.1.1. Investigation of petroleum ether extract (GC/MS)

Twenty-nine compounds were identified in the petroleum ether extract of *A. aestivus* flowers using GC/MS analysis (Table 1) comprising 93.13% of the total extract. The major components were 9,12,15-octadecatrieoic acid methyl ester (26.45%), 9,12-octadecadieroic acid (Z, Z)-methyl ester (18.39%), hexadecanoic acid methyl ester (13.81%), n-hentriacontane (7.12%) and n-pentacosane (5.73%). Among the detected compounds, nine essential oils were reported before in the *A. aestivus* flowers from Cyprus [1] (Table 1). Hexadecanoic acid methyl ester was reported before for its cytotoxicity to human leukemic cells (MOLT-4) and *in vivo* antitumor activity in mice [26-27], in addition to its several pharmacological activities [28].

3.1.2. Investigation of hydromethanolic extract

3.1.2.1. Acid hydrolysis

Investigation of the ethyl acetate fraction on PC showed flavone, flavonol and anthraquinone aglycones. Two dark flavone aglycones (changed to yellow when exposed to ammonia vapor) have PC behavior as apigenin and luteolin standard. Kaempferol (major) and quercetin (minor) were also observed as yellow spots. Two anthraquinone aglycones were observed as red spots, one of them was suggested to be emodin (PC behaviour) which isolated previously from the studied species. Moreover, PC revealed the presence of additional two dark spots unchanged through acid hydrolysis process indicating possible *C*-glycoside structures, whereas, their R_f values and color reaction resembled those of isovitexin and isoorientin standards. Furthermore, some blue spots appeared, two of which matches the R_f value and color reaction of caffeic acid and chlorogenic acid while others are unknown. Glucose was detected as a major sugar moiety in the aqueous fraction.

3.1.2. 2. Identification of the isolated compounds

The first chemical investigation of the hydromethanolic extract of *A. aestivus* flowers led to the isolation of ten phenolic compounds (Fig.1). They were identified as kaempferol (**1**) [29], kaempferol -3-*O*-glucoside (**2**) [30], kaempferol -3,7-di-*O*-glucoside (**3**) [31], luteolin 7-*O*-glucoside (**4**) [32-33],

isoorientin (**5**) [34], isoorientin 7-*O*-glucoside (lutonarin) (**6**) [35], caffeic acid (**7**) [36], emodin (**8**) [37], apigenin 7-*O*-glucoside (**9**) [36] and isovitexin-7-*O*-glucoside (saponarin) (**10**) [38]. Compounds (**2**, **3**, **6-8**) were isolated for the first time from the investigated species while the remaining compounds were reported previously from the aerial parts of *A. aestivus* [33]. In addition, compound (**1**) was reported before in *A. ramosus* [29] and (**4**) was detected in different *Asphodelus* species such as *A. cerasifer* [32] and *A. delphinensis* [33].

Table 1 GC/MS analysis of the petroleum ether extract of *Asphodelus aestivus* flowers

NO	R_t min	Compounds	MW	Mol. formula	Area%
1	17.54	n-Undecane	156	$C_{11}H_{24}$	0.73
2	18.62	Nonanol	144	$C_9H_{20}O$	0.57
3	21.76	n-Dodecane	170	$C_{12}H_{26}$	0.31
4	22.54	Dodecanoic acid,methyl ester	214	$C_{13}H_{26}O_2$	0.64
5	22.82	n-Tetradecane*	198	$C_{14}H_{30}$	0.26
6	25.87	Dodecanol	186	$C_{12}H_{26}O$	0.39
7	26.58	n-Pentadecane*	212	$C_{15}H_{32}$	0.36
8	26.99	Tetradecanoic acid methyl ester	242	$C_{15}H_{30}O_2$	1.23
9	29.04	Isopropyl myristate	270	$C_{17}H_{34}O_2$	0.84
10	31.02	Hexadecanoic acid methyl ester	270	$C_{17}H_{34}O_2$	13.81
11	32.32	n-Hexadecane*	226	$C_{16}H_{34}$	0.37
12	32.86	Ethylene brassylate	270	$C_{15}H_{26}O_4$	0.99
13	34.17	9,12-Octadecadien-oic acid methyl ester	294	$C_{19}H_{34}O_2$	18.39
14	34.30	9,12,15-Octadecatrie-oic acid, methyl ester	292	$C_{19}H_{32}O_2$	26.45
15	34.51	9-Octadecenoic acid (Oleic acid)	282	$C_{18}H_{34}O_2$	0.31
16	34.71	Octadecanoic acid, methyl ester	298	$C_{19}H_{38}O_2$	0.85
17	35.53	n-Eicosane	282	$C_{20}H_{42}$	0.27
18	35.88	n-Docosane*	310	$C_{22}H_{46}$	0.55
19	37.54	n-Tricosane*	324	$C_{23}H_{48}$	3.06
20	38.10	Docosanoic acid, methyl ester	354	$C_{23}H_{46}O_2$	0.33
21	39.14	n-Tetracosane*	338	$C_{24}H_{50}$	1.39
22	40.69	n-Pentcosane*	352	$C_{25}H_{52}$	5.73
23	42.17	n-Hexacosane*	366	$C_{26}H_{54}$	0.80
24	43.61	n-Heptacosane*	380	$C_{27}H_{56}$	3.06
25	45.52	Squalene	410	$C_{30}H_{50}$	1.42
26	46.35	n-Nonacosane	408	$C_{29}H_{60}$	1.46
27	48.67	Ethyl iso-allocholate	436	$C_{26}H_{44}O_5$	0.54
28	48.93	n-Hentriacontane	436	$C_{31}H_{64}$	7.12
29	52.03	Astaxanthin	596	$C_{40}H_{52}O_4$	0.90
Total identified compounds					93.13

*Compounds reported before in the essential oil of *A. aestivus* flowers from Cyprus [1]

3.1.2.3. LC-ESI-MS analysis

The LC-ESI-MS/MS analysis of the hydromethanolic extract of *A. aestivus* flowers (negative ion mode) (Fig. 2) led to the identification of twenty three compounds. The detected compounds were identified by mass fragmentation and their relative retention times compared with the isolated compounds which used as authentic standards and the previously published literature data (peaks 8, 11, 13, 14, 15, 16, 17, 19, 22, 23). Peak 1 (*Rt* 2.4), was proposed as gluconic/galactonic acid which occurs naturally in fruit, honey and wine [39] and demonstrated as an $[M-H]^-$ ion at *m/z* 195 with fragment ions at *m/z* 177, *m/z* 159, *m/z* 129, *m/z* 99 [40]. While peak 2 (*Rt* 2.75) is considered one of the amino acid derivatives confirmed by the presence of a molecular ion peak $[M-H]^-$ at *m/z* 293 and a fragment ion at *m/z* 131 after the loss of glucose moiety with that at *m/z* 87 due to the loss of $H_2O + CO$ so, this compound was identified as aspargine-glucoside [41]. Peak 3 (*Rt* 3.59) is tentative identified as citric/isocitric acid with molecular ion peak at *m/z* 191 supported by the existence of the fragment ion at *m/z* 111 $[M-H–CO_2–2H_2O]^-$ [42]. Quinic acid was presented as peak 5 (*Rt* 4.68) at *m/z* 191, confirmed by the presence of the fragment ion at *m/z* 127 $[M-H-CO-2H_2O]^-$ [43].

Peaks 6 (*Rt* 18.7), 9 (*Rt* 23.8) & 10 (*Rt* 24.22) produced the same molecular ion peak (*m/z* 353) and tentatively identified as derivatives of chlorogenic acid. The fragment ion at *m/z* 191 $[quinic\ acid-H]^-$ and ion at *m/z* 179 $[caffeic\ acid-H]^-$. An additional fragment was shown in peak 10 at *m/z* 173 $[quinic\ acid-H_2O-H]^-$, which is attributed to 4-*O*-caffeoylquinic acid [44]. Thus, peaks 6 and 9 were identified as *cis*- and trans-3-*O*-caffeoylquinic acid, respectively while peak 10 identified as 4-*O*-Caffeoyl quinic acid [36, 44]. The MS spectrum of peak 7 (*Rt* 20.87) showed a molecular ion peak at $[M-H]^-$ at *m/z* 445 and fragment *m/z* 285, indicted the existence of rhein hexoside structure. This suggestion supported by the presence of fragments pattern as that of rhein structure (at *m/z* 267, 211 and 185). Therefore, peak 7 was tentatively identified as rhein-*O*-glucoside based on acid hydrolysis [45]. Peak 12 (*Rt* 30.05) revealed a molecular ion peak $[M-H]^-$ at *m/z* 431 and a base peak ion at *m/z* 269 $[M-H-162]^-$, after loss of glucose moiety. The fragment ion (*m/z* 269) together with additional ions at *m/z* 241$[M-H-CO]^-$ and 225$[M-H-CO_2]^-$ were identical to emodin aglycone structure, so peak 12 is identified as emodin-*O*-glucoside [45]. Peak 18 (*Rt* 43.4) has a molecular ion peak $[M-H]^-$ at *m/z* 623 and produced fragment ions (*m/z* 447 $[M-H-176]^-$), after loss of feruloyl moiety. Another fragment at *m/z* 301 represented as quercetin aglycone after loss of rhamnose unit $[M-H-176-146]^-$. Therefore it tentatively identified as quercetin-

O-feruoyl rhamnoside. Peaks 20 (*Rt* 49.68) and 21 (*Rt* 50.01) pointed to be the oxidation products of oxylipins; the unsaturated fatty acids and showed molecular ion peak $[M-H]^-$ at *m/z* 329 [46] and were proposed to be two of lineolic acids derivatives which distinguished by the existence of a molecular ion at *m/z* 171 [OOC (CH_2)7CH-OH],the neutral loss of 100 amu (329-229) and (311-211) analogous to the loss of an end group HO-CH=CH$(CH_2)3CH_3$ from the oxylipin molecule, so, peak 20 and 21 were identified as trihydroxy octadecenoic acid isomers [47]. Peak 24 (*m/z* 465) was confronted as glycerophosphate lipid which was distinguished by the fragment ion at *m/z* 329 after the neural loss of a dehydrated phosphoglycerol unit (-136 Da), and supported by the presence of the product ion at *m/z* 153. Therefore, it could be identified as trihydroxy octadecenoyl glycerophosphate [48].

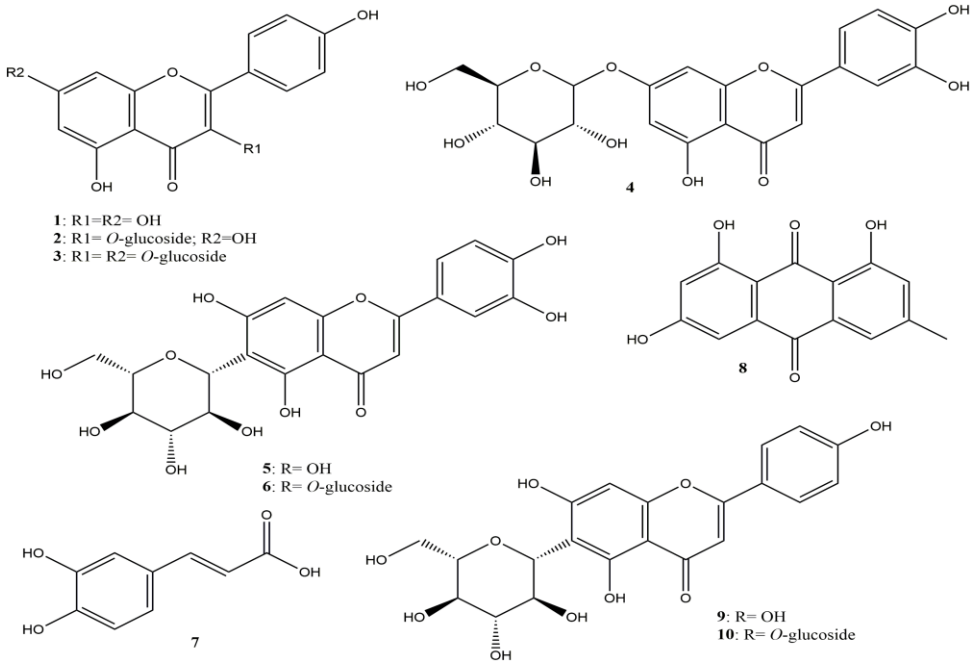

Fig (1): Chemical structure of the isolated compounds from *A. aestivus* flowers.

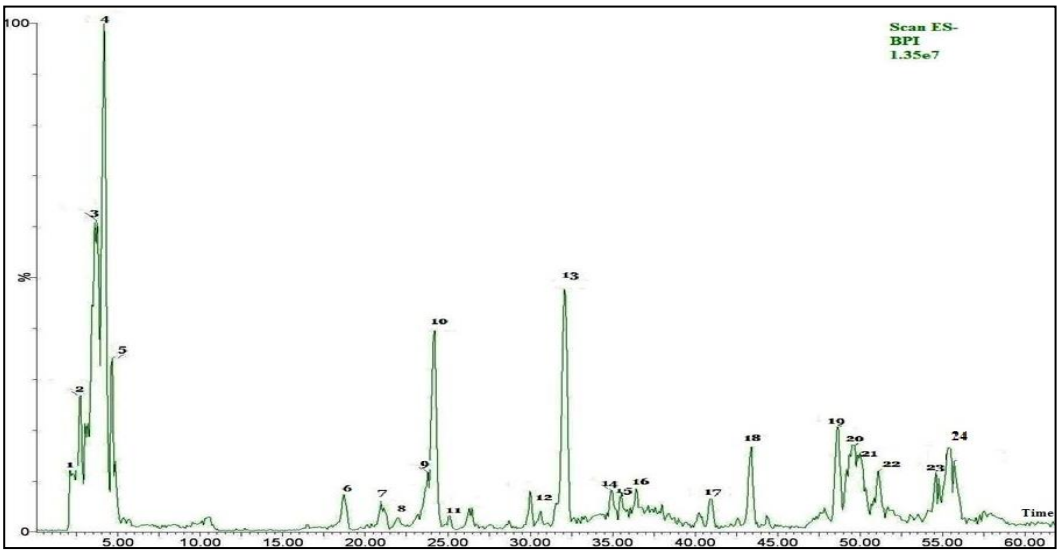

Fig (2): LC-ESI-MS/MS analysis of the hydromethanolic extract of *A. aestivus* flowers

Table 2 Tentative identification of chemical compounds in the hydromethanolic extract of *Asphodelus aestivus* flowers

No.	*Rt* (min)	$[M–H]^-$	*m/z* fragments	Tentative identification	Reference
1	2.4	195	177, 159, 129	Gluconic acid/ galactonic acid	[40]
2	2.75	293	131,113, 87,70	Aspargine-N- glucoside	[41]
3	3.59	191	111	Citric/isocitric acid	[42]
4	4.17	331	97, 79	Unknown	-
5	4.68	191	173, 127,85	Quinic acid	[43]
6	18.7	353	191, 179, 173, 135,127	*cis* 3-Caffeoyl quinic acid	[36]
7	20.87	445	325,285, 241,211, 185	Rhein-*O*- glucoside	[45]
8	21.2	609	447, 357, 327, 285	Isoorientin 7-*O*-glucoside (lutonarin)*	[35]
9	23.8	353	191, 179, 161, 85	*trans* 3-Caffeoyl quinic acid	[36]
10	24.22	353	191, 179, 161, 135	4-*O*- Caffeoyl quinic acid	[36,44]
11	25.1	179	135	Caffeic acid*	[36]
12	30.05	431	269,241,225	Emodin-*O*-glucoside	[45]
13	32.064	447	429,357,327	Isoorientin*	[34]
14	34.98	447	285	Luteolin 7-*O*-glucoside*	[33]
15	35.57	593	431, 341, 311	Isovitexin-7-*O*-glucoside (Saponarin)*	[38]
16	36.41	609	447, 285	Kaempferol -3,7-di-*O*-glucoside*	[30]
17	40.91	447	285	Kaempferol -3-*O*-glucoside*	[29]
18	43.4	623	447, 301, 193, 175, 147	Quercetin-*O*-feruoyl rhamnoside	[18]
19	48.68	285	227,151	Kaempferol*	[28]
20	49.68	329	311, 229,211, 171	Trihydroxyoctadecenoic acid (isomer 1)	[47]
21	50.01	329	311, 229,211, 171	Trihydroxyoctadecenoic acid (isomer 2)	[47]
22	51.18	431	279, 269	Apigenin 7-*O*-glucoside*	[36]
23	54.52	269	241, 225	Emodin*	[37]
24	55.4	465	329, 211, 153	Trihydroxyoctadecenoyl-glycerophosphate	[48]

*Compounds isolated in the present study and used as standard

3.2. Cytotoxic activity

In vitro cytotoxic evaluation of the petroleum ether and aqueous methanol extracts of *A. aestivus* flowers showed moderate inhibition% (63.8 and 68.4) on MCF-7 followed by (52.3% and 65.7%) on HepG2, respectively. Both extracts showed weak inhibition (5.7-14.3%) on HCT-116 and A549. Previous reports of the biological activity of the root and leaf of the dichloromethane and n-hexane fractions of *A. aestivus* showed antitumoral activity against human cancer cells (lung A549 and prostate PC3) through DNA damage [49] while aqueous (infusion and decoction), diethyl ether, ethyl acetate and methanol fractions were evaluated before against breast cancer cell line (MCF-7) and showed that methanol and aqueous extracts exhibited strong cytotoxic activities and all the extracts showed a significant DNA damaging and apoptotic activities [3] but no previous cytotoxic activity was measured for the flower.

The moderate cytotoxic activity *A. aestivus* flower might be related to an active compound or the synergic effect of several detected compounds resulted in the anti-tumoral activity of *A. aestivus* by several reports [3, 50].

4. Conclusion

Total fifty two polar and non-polar compounds were isolated and/or detected from either petroleum ether extract or aqueous methanol extract of *A. aestivus* flowers. Except for nine essential oils and five phenolics, all compounds were reported for the first time from the investigated species. Also, the first cytotoxic evaluation of both extracts showed moderate inhibition% (63.8 and 68.4) on MCF-7 followed by (52.3% and 65.7%) on HEPG-2, respectively.

5. Conflicts of interest

Authors declare that there are no conflicts of interest.

6. Acknowledgments

This work is funded by project number 11010328 in the National Research Centre, Cairo, Egypt.

7. References

[1] K. Polatog˘lu, B. Demirci, K.H. Can Baser, High Amounts of n-Alkanes in the Composition of *Asphodelus aestivus* Brot. Flower Essential Oil from Cyprus, Journal of Oleo Science 65(2016) 867–870.

[2] L. Boulos L. Flora of Egypt, vol 4. Al Hadara Publishing, Cairo, Egypt (2005).
[3] S.Ö. Aslantürk, T.A.Çelik, Investigation of antioxidant, cytotoxic and apoptotic activities of the extracts from tubers of *Asphodelus aestivus* Brot., African Journal of Pharmacy and Pharmacology 7 (2013) 610–621.
[4] I. Çalıs, S.S. Birincio˘glu, H. Kırmızıbekmez, B. Pfeiffer, J. Heilmann, Secondary Metabolites from *Asphodelus aestivus*. Verlag der Zeitschrift f¨ur Naturforschung 61(2006) 1304–1310.
[5] F.B. Caldas, J.C. Moreno Saiz, The International Union for the Conservation of Nature (IUCN) Red List of Threatened Species; The International Union for the Conservation of Nature: Grand, Switzerland (2011).
[6] M. Grieve, A Modern Herbal. Penguin (1984).
[7] G. Usher, A Dictionary of Plants Used by Man. Constable and Company Ltd (1974).
[8] O. Polunin, A. Huxley, Flowers of the *Mediterranean.* Hogarth Press (1987).
[9] A.D. Niebuhr Herbs of Greece. Herb Society of America (1970).
[10] J.C.T. Uphof, Dictionary of Economic Plants. Weinheim (1959).
[11] E. Tuzlaci, P. Aymaz-Eryasar, Turkish folk medicinal plants, Part IV: Gönen (Balıkesir), Fitoterapia 72(2001) 323-343.
[12] E. Ugurlu, O. Secmen, Medicinal plants popularly used in the villages of Yunt Mountain (Manisa-Turkey), Fitoterapia 79 (2008) 126-131.
[13] I. Gurbuz, O. Ustun, E. Yesilada, E. Sezik, N. Akyurek, *In vivo* gastroprotective effects of five Turkish folk remedies against ethanolinduced lesions, Journal of Ethnopharmacology 283(2002) 241-244.
[14] A. Peksel, N. Altas-Kiymaz, S. Imamoglu, Evaluation of antioxidant and antifungal potential of *Asphodelus aestivus* Brot. growing in Turkey, Journal of Medicinal Plants Research 6(2) (2012) 253-265.
[15] K.R. Markham, Techniques of Flavonoid Identification. London: Academic Press (1982).
[16] M.M. Farid, A.Y. Ragheb, M. El-Shabrawy, M.M. Marzouk, S.R. Hussein, A.A.F. Soliman, H. Taha, S.A. Kawashty, GC-MS and LC-ESI-MS analysis of biologically active fractions from *Verbascum letourneuxii*; efficient protocol for *in vitro* propagation, Biocatalysis and Agricultural Biotechnology 29(2020)101817.
[17] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Edition Allured Publishing Corporation, Carol Stream (2007).
[18] E.A. El-Wakil, E.S.S. Abdel-Hameed, M.M. El-Sayed, E.E. Abdel-Lateef, Identification of the chemical composition of the methanolic extract of *Salix tetrasperma* Roxb. using LC-ESI-MS and evaluation its potential as antioxidant agent, Der Pharma Chemica 7(2) (2015)168-177.
[19] S.R. Hussein, A. Elkhateeb, M.M. Marzouk, LF. Ibrahim, S.A. Kawashty, Phytochemical investigation of *Oligomeris linifolia* (Vahl) Macbr. (Resedaceae), Biochemical Systematics and Ecology 49(2013) 73-76.
[20] M.M.Marzouk, Flavonoid constituents and cytotoxic activity of *Erucaria hispanica* (L.) Druce growing wild in Egypt, Arabian Journal of Chemistry (2011).
[21] S.A. Kawashty, S.R. Hussein, M.M. Marzouk, L.F. Ibrahim, M.M.I. Helal, S.I.M. El Negomy, Flavonoid constituents from *Morettia philaena* (Del.) DC. and their antimicrobial activity, Journal of Applied Sciences Research 8 (3) (2012) 1484-1489.
[22] M.E.S. Kassem, M.S. Afifi, M.M. Marzouk, M.A.Mostafa, Two new flavonol glycosides and biological activities of *Diplotaxis harra* (Forssk.) Boiss., Natural Product Research 27(24) (2013) 2272-80.
[23] L.F. Ibrahim, M.M. Marzouk, S.R. Hussein, S.A. Kawashty, K. Mahmoud, N.A.M. Saleh, Flavonoid constituents and biological screening of *Astragalus bombycinus* Boiss., Natural Product Research 27(2013) 386-93.
[24] M.O. El-Shabrawy, M.M. Marzouk, S.A. Kawashty, H.A. Hosni, I.A. El Garf, N.A.M. Saleh, Flavonoid constituents of *Dipcadi erythraeum* Webb. & Berthel, Asian Pacific Journal of Tropical Disease 6(5) (2016) 404-405.
[25] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, Journal of Immunological Methods 65 (1) (1983) 55-63.
[26] H. Harada, U. Yamashita, H. Kurihara, E. Fukushi, J. Kawabata, Y. Kamei Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Research 22 (2002) 2587–2590.
[27] N.A. El Semary, S.M. Ghazy, M.M. Abd El Naby, Investigating the taxonomy and bioactivity of an Egyptian Chlorococcum isolate, Australian Journal of Basic and Applied Sciences 3 (2009) 1540–1551.
[28] P.P. Kumar, S. Kumaravel, C. Lalitha, Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*, African Journal of Biomedical Research 4(2010)191–195.
[29] K.Adawi, Comparison of the Total Phenol, Flavonoid Contents and Antioxidant Activity of Methanolic Roots Extracts of *Asphodelus microcarpus* and *Asphodeline lutea* Growing in Syria, International Journal of Pharmacognosy and Phytochemical Research 9(2017)159–164.
[30] M.M. Marzouk, S.R. Hussein, A. Elkhateeb, M.M. Farid, L.F. Ibrahim, E.S. Abdel-Hameed, Phenolic profiling of *Rorippa palustris* (L.) Besser (Brassicaceae) by LC-ESI-MS: chemosystematic significance and cytotoxic activity, Asian Pacific Journal of Tropical Disease 6(2016):633–637.
[31] M.A. Salem, M.M. Farid, M. El-Shabrawy, R. Mohammed, S.R. Hussein, M.M. Marzouk, Spectrometric analysis, chemical constituents and cytotoxic evaluation of *Astragalus sieberi* DC. (Fabaceae), Scientific African 7(2020): e00221.
[32] C.A.Williams, Biosystematics of the Monocotyledoneac—Flavonoid Patterns in Leaves of the Liliaceae, Biochemical Systematics and Ecology 3(1975) 229–244.
[33] M.O. El Shabrawy, M.M. Marzouk, S.A. Kawashty, H.A. Hosni, I.A. El Garf , N.A. Saleh, A chemosystematic study of *Asphodelus aestivus* Brot. (Asphodelaceae) in Egypt, Egyptian Pharmaceutical Journal 17(2018) 150–154.
[34] M. Farid, S. Hussein, A. Trendafilova, M. Marzouk, A. El Oqlah, M. Saker, Phytochemical constituents of the butanol fraction of *Arum palaestinum* Boiss.:

cytotoxic and antiviral screening, Materials and Environmental Science 8(7) (2017) 2585-2591.

[35] M.M. Marzouk, A. Elkhateeb, R.R. Abdel Latif, E.S. Abdel-Hameed, S.A. Kawashty, S.R. Hussein, C-glycosyl flavonoids-rich extract of *Dipcadi erythraeum* bulbs:phytochemical and anticancer evaluations, Journal of Applied Pharmaceutical Science 9(6) (2019) 94–98.

[36] M.M. Marzouk, S.R. Hussein, A. Elkhateeb, M. El-shabrawy, E.S. Abdel-Hameed S.A. Kawashty, Comparative study of *Mentha* species growing wild in Egypt: LC-ESI-MS analysis and chemosystematic significance, Journal of Applied Pharmaceutical Science 8(08) (2018) 116-122.

[37] J. Zhang, W. Gao, Z. Liu, Z. Zhang, Identification and Simultaneous Determination of Twelve Active Components in the Methanol Extract of Traditional Medicine Weichang'an Pill by HPLC-DAD-ESI-MS/MS, Iranian Journal of Pharmaceutical Research 12(1) (2013) 15-24.

[38] S.R. Hussein, R.R. Abdel Latif, M.M. Marzouk, et al. Spectrometric analysis, phenolics isolation and cytotoxic activity of *Stipagrostis plumosa* (Family Poaceae). Chemical Papers 72(2018) 29–37.

[39] S. Ramachandran, P. Fontanille, A. Pandey, C. Larroche, Fed-batch production of gluconic acid by terpene-treated *Aspergillus niger* spores, Applied Biochemistry and Biotechnology 151(2008) 413–423.

[40] D.F. Felipe, L.Z.S. Brambilla, C. Porto, E.J. Pilau, D.A.G.Cortez, Phytochemical Analysis of *Pfaffia glomerata* Inflorescences by LC-ESI-MS/MS, Molecules 19 (10) (2014) 15720-15734.

[41] M. Chikasou, S. Inohana, T. Yokozeki, H. Tuchiya, K. Fujita, Development of LC-MS and LC-MS/MS methods for free asparagine in grains, Journal of Food Hygienic Socity of Japan 59(2018)248–256.

[42] M. Emam, P.E. Moustafa, A. Elkhateeb, S.R. Hussein, M.M. Marzouk, S.S. Abd El-Rahman, E.S. Abdel-Hameed, R.F. Abdel-Rahman, *Dobera glabra* (Forssk.) Poir. (Salvadoraceae); phenolic constituents of the aqueous leaves extract and evaluation of its anti-inflammatory, analgesic activities, Heliyon 7 (2) (2021) e06205.

[43] A. Taamalli, D. Arráez-Román, L. Abaza, I. Iswaldi , A. Fernández-Gutiérrez, M. Zarrouk, A. Segura-Carretero, LC-MS-based metabolite profiling of methanolic extracts from the medicinal and aromatic species *Mentha pulegium* and *Origanum majorana*, Phytochemical Analysis 26 (2015) 320-330.

[44] E.N. Ncube, M.I. Mhlongo, L.A. Piater, P.A. Steenkamp, I.A. Dubery, N.E. Madala, Analyses of chlorogenic acids and related cinnamic acid derivatives from Nicotiana tabacum tissues with the aid of UPLC-QTOF-MS/MS based on the in-source collision-induced dissociation method, Chemistry Central Journal 8(2014) 66.

[45] M. Ye, J. Han, H. Chen, J. Zheng, Analysis of phenolic compounds in rhubarbs using liquid chromatography coupled with electrospray ionization mass spectrometry, Journal of the American Society for Mass Spectrometry 18 (2007) 82–91.

[46] T. Pussa, P. Raudsepp, P. Toomik, R. Pallin, U. Maeorg, S. Kuusik, R. Soidla, M. Rei, Study of oxidation products of free polyunsaturated fatty acids in mechanically deboned meat, Journal of Food Compostion and Analysis 22 (2009)307-314.

[47] A. Napolitano, A. Cerulli, C. Pizza, S. Piacente, Multi-class polar lipid profiling in fresh and roasted hazelnut (Corylus avellana cultivar “Tonda di Giffoni”) by LC-ESI/LTQOrbitrap/MS/MSn, Food Chemistry, 269 (2018) 125-135.

[48] M. Pulfer, R.C. Murphy, Electrospray mass spectrometry of phospholipids, Mass Spectrometry Reviews 22(5) (2003) 332-64.

[49] A. Al Groshi, L. Nahar, E. Andrew, A. Auzi, S.D. Sarker, F.M.D. Ismail, Cytotoxicity of *Asphodelus aestivus* against two human cancer cell lines, Natural Products Chemistry and Research 5 (2017).

[50] S.M. Abu-darwish, M. Ateyyat, The Pharmacological and Pesticidal Actions of Naturally Occurring 1, 8-dihydroxyanthraquinones Derivatives, World Journal of Agricultural Sciences 4 (2008) 495–505.