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Isoscutellarein 8, 4′-Dimethyl ether glycosides as cytotoxic agents and chemotaxonomic markers in *Kickxia aegyptiaca*

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ABSTRACT

Two new isoscutellarein methyl ether derivatives were isolated and identified from *Kickxia aegyptiaca* (L.) Nábělek; isoscutellarein 8, 4′- di-OCH_3 7-*O*-rutinoside-4‴-acetate; isolinariin **(1)** and isoscutellarein 8, 4′-di-OCH_3 7-*O*-rutinoside; isopectolinarin (2), together with eight known flavonoid compounds; ladenine (**3**), pectolinarigenin (**4**), pectolinarin (**5**), linariin (**6**), pectolinarigenin 7-*O*-robinobioside (**7**), scutellarein 7-*O*-rutinoside (**8**), scutellarein 4′-OCH_3-7-*O*-rutinoside (**9**) and linarin (**10**). These structures were elucidated on the basis of chromatographic and spectral analysis. Furthermore, the LC-ESI-MS technique was performed and revealed the tentative identification of trihydroxy-dimethoxy flavone glycosides for the first time. In addition, the plant extract and the isolated flavonoids were screened against two human tumor cell lines; breast (MCF-7) and colon (HCT-116). Compound (**4**) showed the highest % of cytotoxicity at100 μg/ml with 91.1 and 37.9 against MCF-7 and HCT-116, respectively, while compounds **1**, **2**, **3** and **5** showed moderate activities against MCF-7 as 68.2%, 57.5%, 65.8% and 58.8%, respectively. Also, the chemotaxonomic study revealed that *K. aegyptiaca* is the most advanced species in tribe Antirrhineae and is closely related chemically to *Veronica* species.

1. Introduction

The genus *Kickxia* Dumort. (Plantaginaceae) is comprised about 10 accepted species (The Plant List, 2013) and containing numerous medicinal species commonly known as cancerworts or fluellins (Jan and Khan, 2016). These species are distributed in North Africa, South-Central and South-Western Asia, Europe and Macaronesia (Ghebrehiwet, 2001). According to Täckholm (1974), *Kickxia* is the largest genus of family Scrophulariaceae in Egypt and being represented by 12 species. The numbers of species are reduced to 9 species according to Boulos (2002, 2009). *Kickxia aegyptiaca* (L.) Nábělek [Syn: *Antirrhinum aegyptiacum* L., *Linaria aegyptiaca* (L.) Dum. Cours. and *Elatinoides aegyptiaca* (L.) Wettst,] is a perennial plant grows wild in Egypt in sand plains, wadi beds of the northern and eastern deserts, Sinai, the Mediterranean coastal lands and the Oases (Boulos, 2009).

Previous phytochemical investigations of *K. aegyptiaca* led to the isolation of iridoids and flavonoids (Ferhat et al., 2010). The plant methanol extract showed weak antioxidant properties and moderate to weak cytotoxic activity (Moustafa et al., 2014a, b).

Our present species was firstly known to Linnaeus (1753), who placed it in his generally circumscribed *Antirrhinum* and was treated as *A.* aegyptiacum. On the bases of capsule dehiscence mode, it was treated by Chavannes (1833) who placed it within the genus *Linaria* (sect. *Elatinoides*) and was circumscribed as *L. aegyptiaca*. Formerly, it has been circumscribed in the group *"Operculatae"* within the genus *Elatinoides* (Wettstein, 1891) and named as *E. aegyptiaca*. The classification as the genus *Elatinoides* was then used by subsequent authors until Janchen (1933) who transferred several species of the genus *Elatinoides* to the genus *Kickxia* and divided it into two sections; *K.* sect. *Kickxia* (which *K. aegyptiaca* belongs) and *K.* sect. *Valvatae*. This classification has been adopted by Nábelek (1926), Tackholm (1974) and Sutton (1988). Ghebrehiwet (2001) considered two genera in *Kickxia s.l.*; *Kickxia* Dumort *s. s.* (which *K. aegyptiaca* belongs) and *Nanorrhinum* Betsche.

The present study aims to investigate the phytochemical constituents of *K. aegyptiaca* with the evaluation of its chemotaxonomy and evaluating the anticancer activity of the extract and the isolated compounds.

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2. Material and method

2.1. General

1D and 2D NMR experiments (^{1}H, ^{13}C, HSQC and HMBC) were recorded on Bruker spectrometer: 400 MHz (^{1}H NMR), 100 MHz (^{13}C NMR). UV spectrophotometer (Shimadzu UV-240). Mass spectrometer (Waters Corporation, Milford, MA01757 U.S.A). PC (descending) Whatman No. 1 and 3 MM papers, using solvent systems (1) H_2O, (2) 15% HOAc (H_2O–HOAc 85:15), (3) BAW (*n*-BuOH–HOAc–H_2O 4:1:5, upper layer), (4) (C_6H_6–*n*-BuOH–H_2O–pyridine 1:5:3:3, upper layer). Solvents 3 and 4 were used for sugar analysis, Sephadex LH-20 (Pharmazia). Authentic samples were obtained from the department of phytochemistry and plant systematics, NRC. Complete acid hydrolysis for *O*-glycosides (2 N HCL, 2 h, 100 °C) were carried out and followed by paper co-chromatography with authentic samples to identify the aglycones and sugar moieties.

2.2. Plant material

K. aegyptiaca was collected from Northern coast in February 2016. The sample was identified by Prof. Dr M.M.Marzouk. A voucher specimen (No. M935) has been deposited in the herbarium of the National Research Centre (CAIRC), Giza, Egypt.

2.3. Extraction and isolation

The aerial parts of *K. aegyptiaca* were air dried and ground in the laboratory, and the obtained powder (1400 g) was extracted with CH_2Cl_2: MeOH (1: 1) three times at room temperature. The extract was evaporated under reduced pressure and temperature to obtain a residue of 300 g, then fractionated on CC silica gel 60 (230–400 mesh, Merck, Darmstadt, Germany) (6 × 120 cm) eluting with 100% $CHCl_3$ then $CHCl_3$/MeOH up to 100% MeOH. Eighteen $CHCl_3$/MeOH fractions were monitored by TLC (Merck, Darmstadt, Germany, Kieselgel 60 F254, 0.25 mm) eluting with $CHCl_3$:H_2O:MeOH (50:3:1 and 30:3:1) to give five main fractions. Each fraction was subjected to paper chromatography (3 MM) by using BAW (n-BuOH: HOAc: H_2O, 4:1:5, upper phase) as solvent and each individual compound was subjected to a Sephadex LH-20 column (Pharmazia, Uppsala, Sweden) with methanol as eluent for the final purification (Mabry et al., 1970).

Compound **1**. Yellow amorphous powder. UV spectral data, λ_{max} (nm): (MeOH) 272, 334; (+NaOMe) 283,332sh, 401; (+$AlCl_3$) 287, 304, 346, 368sh; (+$AlCl_3$/HCl) 279, 305, 343, 368sh; (+NaOAc) 281, 332; (+NaOAc/H_3BO_3) 281, 335. The negative-ion ESIMS [M-H]$^-$ *m/z* 663.1, molecular formula $C_{31}H_{36}O_{16}$. ^{1}HNMR (DMSO-d6, 400 MHz): δ 1.09 (3H, d, J = 5.8 Hz, H6‴), 1.71 (3H, s, CH_3CO), 3.78 (1H, s, 4′-OCH_3), 3.86 (1H, s, 8-OCH_3), 4.59 (1H, J = 2 Hz, H-1‴), 5.00 (1H, d, J = 7.2 Hz, H1″), 6.33 (1H, s, H-6), 6.66 (1H, s, H-3), 7.14 (2H, d, J = 8.0 Hz, H-3′,5′), 7.98 (2H, d, J = 8.0 Hz, H-2′,6′), 12.90 (1H, s, 5-OH). ^{13}C NMR (DMSO-d6, 100 MHz): δ 163.94 (C-2), 104.1 (C-3), 182.2 (C-4), 152.2 (C-5), 99.8 (C-6), 154.4 (C-7), 127.8 (C8), 156.6 (C-9), 105.9 (C-10), 122.7 (C-1′), 128.9 (C2′,6′), 115.2 (C-3′,5′), 162.2 (C-4′), 56.1 (4′-OCH_3), 56.2 (8-OCH_3), 100.2 (C-1″), 73.5 (C-2″), 76.1(C-3″),70.1(C-4″),75.9(C-5″),66.5(C6″), 99.9 (C-1‴), 70.8 (C-2‴), 68.8 (C-3‴), 73.7 (C4‴), 65.8 (C-5‴), 17.4 (C-6‴), 21.3 (**C**H_3CO), 170.6 (CH_3**C**O).

Compound **2**. Yellow amorphous powder. UV spectral data, λ_{max} (nm): (MeOH) 272, 336; (+NaOMe) 281,332sh, 403; (+$AlCl_3$) 287, 305, 344, 370sh; (+$AlCl_3$/HCl) 282, 304, 343, 369sh; (+NaOAc) 279, 334; (+NaOAc/H_3BO_3) 281, 336. The negative-ion ESIMS [M-H]$^-$ *m/z* 621.2, molecular formula $C_{29}H_{34}O_{15}$. ^{1}HNMR (DMSO-d6, 400 MHz): δ1.12 (3H, J = 6 Hz, H-6‴), 3.69 (1H, s, 4′-OCH_3), 3.85 (1H, s, 8-OCH_3), 4.6 (1H, J = 2 Hz, H-1‴), 5.00 (1H, d, J = 7.2 Hz, H1″), 6.31 (1H, s, H-6), 6.63 (1H, s, H-3), 7.14 (2H, d, J = 8.0 Hz, H-3′,5′), 7.99 (2H, d, J = 8.0 Hz, H-2′,6′).^{13}C NMR (DMSO-d6, 100 MHz): δ 163.8 (C-2), 104.0 (C-3), 182.1 (C4), 152.3 (C-5), 99.9 (C-6), 152.5 (C-7), 128.7 (C-8), 156.4 (C-9), 105.8 (C-10), 122.9 (C-1′), 128.9 (C2′,6′), 115.2 (C-3′,5′), 162.2 (C-4′), 56.1 (4′-OCH_3), 56.3 (8-OCH_3), 100.4 (C-1″), 73.5 (C-2″),76.4(C-3″),69.7(C-4″),76.1(C-5″),65.9(C6″), 100.2 (C-1‴), 70.8 (C-2‴), 70.1 (C-3‴), 71.9 (C4‴), 68.8 (C-5‴), 18.1 (C-6‴).

2.4. LC-ESI-MS analysis

LC-ESI-MS analysis was carried out on a XEVO TQD triple quadruple instrument and mass spectrometer (Waters Corporation, Milford, MA01757 U.S.A). The sample (100 μg/mL) solution was prepared using HPLC analytical grade solvent of MeOH, filtered using a membrane disc filter (0.2 μm) then subjected to LC-ESI-MS analysis. Samples injection volumes (10 μL) were injected into the UPLC instrument equipped with reverse phase C-18 column (ACQUITY UPLC - BEH C18 1.7 μm particlesize - 2.1 × 50 mm Column). Sample mobile phase was prepared by filtering using 0.2 μm filter membrane disc and degassed by sonication before injection. Mobile phase elution was made with the flow rate of 0.2 mL/min using gradient mobile phase comprising two eluents: eluent A (0.1% FA in H_2O) and eluent B) (0.1% FA in MeOH). The parameters for analysis were carried out using negative ion mode as follows: source temperature 150 °C, cone voltage 30 eV, capillary voltage 3 kV, desolvation temperature 440 °C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h. Mass spectra were detected in the ESI between *m/z* 100–1000. The peaks and spectra were processed using the Maslynx 4.1 software. The flavonoids isolated in the present study together with other pure flavonoids obtained from Phytochemistry and Plant Systematics department were used as authentics(Marzouk, 2010;Hussein, 2017; Kawashty, 2012; Marzouk, 2012Hussein et al., 2018). Known peaks were identified by comparing their retention time and mass spectrum with the isolated flavonoids in the present study. Unknown peaks were tentatively identified by comparing their mass fragmentation pattern with literatures.

2.5. Cell lines

Human breast carcinoma (MCF-7 cell line) and colon carcinoma (HCT-116 cell line) were obtained from Karolinska Centre, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden.

2.6. Cell viability assay

This was done according to Batran, 2018 as described by (Mosmann, 1983). Following culturing for 10 days, the cells were seeded at concentration of 10 × 103 cells per well in case of MCF-7, 20 × 103 cells/well in a fresh complete growth medium in case of HCT-116 at 37 °C for 24 h in water jacketed carbon dioxide incubator. After 48 h' incubation, the medium was aspirated and then 40 μl MTT salt (2.5 mg/ml) was added for further 4 h 200 μl 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37 °C. The absorbance was measured at 595 nm 100 μg/ml doxorubicin was used as positive control and 0.5% DMSO was used as negative control. 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. Identification of the isolated compounds and LC-ESI-MS analysis

Two new flavonoid compounds; isoscutellarein 8, 4′- di-OCH_3 7-*O*-rutinoside -4‴-acetate; isolinariin (**1**) and isoscutellarein 8, 4′- di-OCH_3-7-*O*-rutinoside; isopectolinarin (**2**) were isolated from *K. aegyptiaca* extract along with eight known flavonoid compounds (Fig. 1). These were identified as ladenine; scutellarein 7, 4′ di-OCH_3 (**3**) (Ferhat et al.,

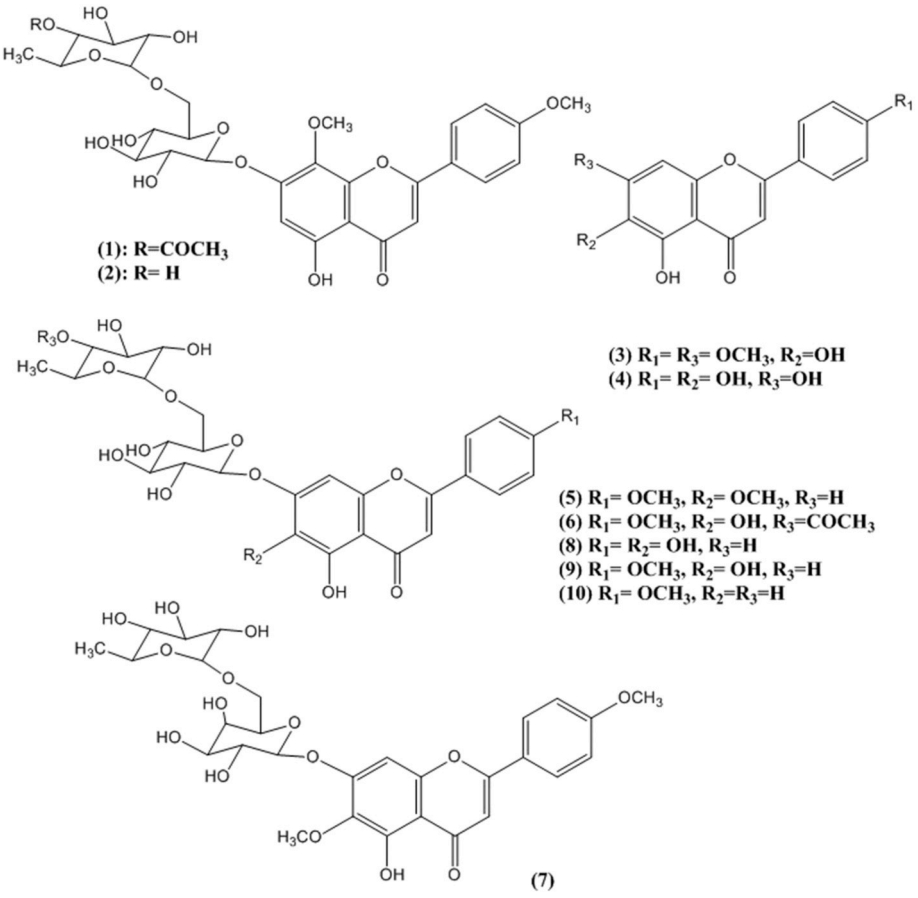

Fig. 1. Chemical structure of isolated flavonoids from *K. aegyptiaca*.

2010), pectolinarigenin; scutellarein 6,4′ di- OCH_3 (**4**) (Singh and Parakash, 1987), pectolinarin; pectolinarigenin 7-*O*-rutinoside (**5**) (Ahmad et al., 2006), linariin; pectolinarin 4‴-acetate (Ostuka, 1992) (**6**), pectolinarigenin 7-*O*-robinobioside (**7**), scutellarein 7-*O*-rutinoside (**8**), scutellarein 4′-OCH_3-7-*O*-rutinoside (**9**) (Grayer et al., 2002) and linarin; acacetin 7-rutinoside (**10**) (Ostuka, 1992). Their structure elucidation was carried out through R_f values, colour reactions, chemical investigations (complete acid hydrolysis) and physical investigations (UV, NMR and MS) (Agrawal and Bansal, 1989; Mabry et al., 1970; Markham, 1982; Markham and Geiger, 1994). Spectral data of the known flavonoids were in good accordance with those previously published.

Compound **1** was isolated as a yellow amorphous powder. UV spectral data with diagnostic shift reagents suggested the presence of a 7, 4′-disubstituted flavone with free hydroxyl group at position 5 and absence of any orthodihydroxy group (Mabry et al., 1970; Markham, 1982). Complete acid hydrolysis (2 N HCl, 2 h, 100 °C) yielded glucose and rhamnose as sugar moities (Co-PC) and isoscutellarin 8,4′-dimethyl ether (UV, EIMS and ^{1}H NMR). The negative-ion ESIMS showed a molecular ion peak $[M\text{-}H]^-$at *m/z* 663.1, corresponding to a molecular formula $C_{31}H_{36}O_{16}$. The ^{1}H NMR spectrum showed two pairs of doublets at δ 7.98 (J = 8.0 Hz) and δ 7.14 (J = 8.0 Hz) assigned to H 2′,6′ and H 3′, 5′, respectively. The doublet signal of H3′,5′ was appeared with a downfield ~0.4 ppm, indicated the substitution of hydroxyl group at position 4′ (Markham and Geiger, 1994; Hussein et al., 2013). Two singlets were also shown at δ 6.66 and δ 6.33 are assigned to H-3 and H-6, respectively. The downfield chemical shift of H-6 confirmed that C-7 is substituted in ring A (Mabry et al., 1970; Markham and Geiger, 1994; Kassem, 2013). Additionally two OCH_3 groups are shown at δ 3.78 and δ 3.86. The ^{1}H NMR spectrum also revealed two distinct anomeric proton resonances at δ 5.00 (J = 7.2 Hz), and δ 4.59 (J = 2 Hz), attributed to H-1″ and H-1‴ of the β-glucopyranose and α-rhamnopyranose units, respectively (Markham and Geiger, 1994). H-6‴ of the rhamnose moiety appear as doublet at δ 1.09 (J = 5.6 Hz). The signals at δ 4.59 and 1.09 are characteristic of rhamnose in rutinoside structure (Markham and Geiger, 1994). Acetyl protons were appeared as singlet at δ 1.71. The ^{13}C NMR spectrum displayed 31 carbon resonances; 17 of which were assigned to the aglycone moiety, 12 to the rutinose unit and 2 for the acetyl group. A significant downfield shift was observed at the 4‴ position (~1.67 ppm), and upfield shifts were observed at both sides of carbon signals (~3.2 1 and~ 2.14 for the C3‴ and C5‴, respectively). This was indicative that the acetyl group was attached to the OH group at the 4‴ position (Markham and Geiger, 1994). The HSQC experiment showed the two anomaric protons of glucose and rhamnose moieties at δ 5.00 and δ 4.59 were correlated with δ 100.2 and 99.9, respectively. Additionally the two OCH_3 at δ 3.78 and δ 3.86 with δ56.1 and 56.2, respectively and the protons in CH_3 of the acetyl group at δ 1.71 with δ 21.3. In the HMBC spectrum, the anomeric proton of -the glucopyranosyl unit (H-1″, δ 5.00) showed a correlation with C-7 (δ 157.2), and that of the rhamnosyl unit (H-1‴, δ 4.59) is correlated with C-6″ (δ 66.5). The two OCH_3 at δ 3.78 and δ 3.86 are correlated with δ 127.8 and 162.2, which attributed to C8 and C4', respectively. Additionaly, the protons in CH_3 of the acetyl group at δ 1.71 are cross correlated with δ 170.6 (C═O of the acetyl group) and δ 73.7 (C-4‴). On the basis of the previously discussed data, compound 1 was identified as isoscutellarein 8, 4′- di-OCH_3 7-*O*-rutinoside-4‴-acetate (isolinariin). Chemical and spectral analysis of compound **2** showed consistence with the presence of compound **1** laking the acetyl group and identified as isoscutellarein 8, 4′- di-OCH_3 7-*O*-rutinoside (isopectolinarin).

The ten isolated flavonoids were used as authentic samples and were observed in the chromatogram as peaks **8**, **10**, **11**, **12**, **14**, **15**–**19** (Fig. 2,

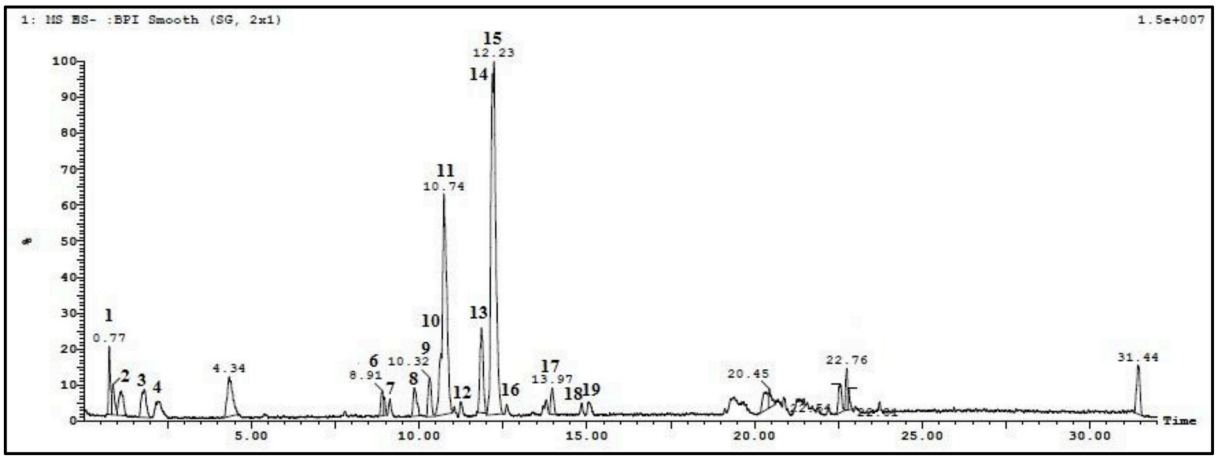

Fig. 2. LC–ESI–MS analysis of phenolic compounds in *K. aegyptiaca* extract.

Table 1
LC–ESI–MS analysis of phenolic compounds in *K. aegyptiaca* extract.

Peak No. (isolate No.)	*Rt* (min)	M	$[M-H]^-$	*m/z* fragments	identification
1 (–)	0.77	472	471	179, 135, 119	Caffeic acid-*O*-dirhamnoside
2 (–)	0.88	356	355	193, 179, 133	Ferulic acid -*O*-hexoside
3 (–)	1.12	404	403	389, 373, 359, 343	Dihydroxy-pentamethoxyflavone
4 (–)	1.80	492	491	461, 431, 401	Unknown
5 (–)	4.34	342	341	179	Caffeic acid-*O*-hexoside
6 (–)	8.91	638	637	329, 314, 301	Trihydroxy-dimethoxy flavone-*O*-rutinoside
7 (–)	8.97	680	679	637, 329	Trihydroxy-dimethoxy flavone-*O*-rutinoside acetate
8 (**9**)	9.86	608	607	299, 285	Scutellarein 4′-OCH_3-7-*O*-rutinoside
9 (–)	10.32	680	679	637, 329, 314, 301	Trihydroxy-dimethoxy flavone-*O*-rutinoside acetate
10 (**5**)	10.71	622	621	313, 299, 285	Scutellarein 6, 4′- di-$OCH_3$7-*O*-rutinoside (Pectolinarin)
11 (**2**)	10.74	622	621	313, 299, 285	Isoscutellarein 8, 4′-di-$OCH_3$7-*O*-rutinoside (isopectolinarin)
12 (**7**)	11.38	622	621	313, 299, 285	Pectolinarigenin 7-*O*-robinobioside
13 (–)	11.86	706	705	663, 621, 313, 299, 285	Linariin-*O*-acetate
14 (**1**)	12.18	664	663	621, 313, 299, 285	Isoscutellarein 8, 4′- di-$OCH_3$7-*O*-rutinoside-4‴-acetate (isolinariin)
15 (**6**)	12.23	664	663	621, 313, 299, 285	Scutellarein 6, 4′- di-$OCH_3$7-*O*-rutinoside-4‴-acetate (linariin)
16 (**8**)	13.81	594	593	285	Scutellarein 7-*O*-rutinoside
17 (**10**)	13.97	592	591	283, 269	Acacetin 7-*O*-rutinoside (linarin)
18 (**3**)	14.5	314	313	299, 285	Scutellarein 7, 4′ di- OCH_3 (Ladenine)
19 (**4**)	15.17	314	313	299, 285	Scutellarein 6, 4′- di OCH_3 (Pectolinarigenin)

Table 1). Other nine additional peaks were also observed and represented as peaks 1–7, 9 and 13. Two caffeic acid derivative (peaks 1 and 5) with $[M-H]^-$ ion at *m/z* (471 and 341) were tentatively characterized as caffeic acid-*O*-dirhamnoside and caffeic acid-*O*-hexoside, respectively based on the loss of dirhamnosyl (−292) and hexoside (−162) moieties and the presence of characteristic fragments at *m/z* 179 and *m/z* 135 (Spínola et al., 2015). Peak 2 with $[M-H]^-$ ion at *m/z* 355 and fragmentation pattern *m/z* *m/z* 193 [M-H-hexose]$^-$ and 149 [M-H-hexose-COOH]$^-$, indicating the presence of ferulic acid -*O*-hexoside (Marzouk et al., 2019). Peak 3 showed a molecular ion peak at *m/z* 403 $[M-H]^-$ with mass fragments *m/z* 389 [M-H- CH_3]$^-$, 373 [M-H- $2CH_3$]$^-$, 359 [M-H-$3CH_3$]$^-$and 343 [M-H-$4CH_3$]$^-$ and tentatively identified as a dihydroxy-pentamethoxyflavone (Zhou et al., 2009).

Peak 6 showed a molecular ion peak at *m/z* 637 $[M-H]^-$ and revealed a fragment at *m/z* 329 [M-H-308]$^-$, suggesting the neutral loss of rutinoside moiety. The presence of other fragments at *m/z* 315 [M-H-308-14]$^-$and 301 [M-H-308-28]$^-$, indicating the presence of a dimethoxy-trihydroxy flavone structure. Thus, peak 6 was tentatively identified as trihydroxy-dimethoxy flavone-*O*-rutinoside.

Also, peaks 7 and 9 showed the same fragmentation pattern as peak 6 and had a molecular ion peak with an extra 42 amu (*m/z* 679), suggestive for an acetyl group connected to a rutinoside moiety. Accordingly, peaks 7 and 9 were tentatively identified as two isomers of trihydroxy-dimethoxyflavone -*O*-rutinoside acetate.

Peak 13 showed fragmentation patterns similar to peak 15 (compound **6**) and revealed a molecular ion peak at *m/z* 705 $[M-H]^-$ with an extra 42 amu, indicating the linkage of additional acetyl group to the rutinoside group. Therefore, peak 13 was tentatively identified as one of isolinariin isomers (C, D or E) which are previously reported from *Linaria japonica* (Widyowati et al., 2016).

3.2. Cytotoxic evaluation

The plant extract and the isolated compounds were screened as anti-proliferative agents on two human tumor cell lines; breast (MCF-7) and colon (HCT-116) at concentration 100 μg/ml and the cytotoxic potentiality of treated cells were compared with untreated one. The results revealed that pectolinarigenin (4) possessed highest cytotoxic activity on MCF-7 with inhibition 91.1%; this results are supported by previous study which stated that pectolinarigenin showed potent anti-proliferation activity by inducing apoptosis and downregulation of Bcl 2 expression in MCF-7 breast cancer cell. (Lu et al., 2014). Moreover, isolinariin (1), isopectolinarin (2), ladenine (3) and pectolinarin (5) showed moderate inhibition for MCF-7 cell line as 68.2%, 57.5%, 65.8% and 58.8%, respectively. The remaining compounds and dichloromethane extract showed moderate to weak activity ranged from 37.8 to 2.2% on MCF-7 cell line. In previous reports, pectolinarigenin and pectolinarin showed potent inhibitory activities on melanogenesis (Lee et al., 2017) and exhibited powerful anti-diabetic, hepatoprotective and

Table 2
In vitro cytotoxic screening ofthe plant extract and isolated flavonoids from *K. aegyptiaca* at 100 μg/ml.

Sample	% of cytotoxicity at 100 μg/ml	
	HCT-116	MCF-7
Isoscutellarein 8, 4′- di-$OCH_3$7-*O*-rutinoside -4‴-acetate (**1**)	18.1	68.2
Isoscutellarein 8, 4′- di-$OCH_3$7-*O*-rutinoside (**2**)	18.1	57.5
Ladenine (**3**)	18.3	65.8
Pectolinarigenin (**4**)	37.9	91.1
Pectolinarigenin 7-*O*-rutinoside (**5**)	14.4	58.8
Pectolinarin 4‴-acetate (**6**)	24.6	20
Pectolinarigenin 7-*O*-robinobioside (**7**)	30.2	11.7
Scutellarein 7-*O*-rutinoside (**8**)	22.6	30.5
Isoscutellarein 4′- $OCH_3$7–*O*-rutinoside (**9**)	34.7	37.8
Linarin (**10**)	32.8	19.4
Plant Extract	14.4	21.6

MCF-7 breast carcinoma. HCT-116 colon.

anticancer activities *in vitro* (Liao et al., 2010; Yoo et al., 2008; Tundis et al., 2005). Regarding the cytotoxic screening of the tested samples on HCT-116; colon carcinoma cells, the inhibition ranged from moderate to weak (37.9–14.4%) (Table 2).

3.3. Chemosystematic significance

Scrophulariaceae s.l. Juss. is a very diverse and heterogeneous family many members of it were recognized into separate families (Tank et al., 2006; Mourad et al., 2015); one of these families is Plantaginaceae Juss. (12 tribe, 92 genera). Tribe Antirrhineae Dumort. is belonging to Plantaginaceae and contains 29 genera and divided into four to six groups on the bases of morphological, plastid *ndhF* sequence data and nuclear marker *ITS* (Ghebrehiwet et al., 2000; Vargas et al., 2004; Fernández--Mazuecos et al. 2013).

Kickxia, Antirrhinum, Linaria are genera of tribe Antirrhineae which are distributed in Egypt, formerly, these genera are known to be synonym to each other but recently, each of them is characterized as a separate genus. From the chemical point of view 10 flavone compounds were isolated and identified from *K. aegyptiaca*, while the 8-hydroyflavone derivative compounds (isoscutellarein nucleus, compound **1, 2**) were not detected before in *Antirrhinum* and *Linaria* while they were isolated from *Veronica liwanensis* K. Koch and *V. longifolia* L. (Plantaginaceae, Veroniceae) (Albach et al., 2003) and *Gratiola officinalis* L. (Plantaginaceae, Gratioleae) (Grayer-Barkmeijer and Tomas-Barberan, 1993). The 6-hydroy flavone derivative compounds (scutellarein nucleus, compounds **3**–**9**) were characterized in *K. aegyptiaca* and some *Linaria* species (Harborne, 1971; Smirnova et al., 1974) while lacked in *Antirrhinum.*

In addition, genus *Antirrhinum* is characterized by presence of flavonol compounds of kaempferol and quercetin with sugar substitution at position 3 and/or 3, 7; flavone compounds of apigenin, luteolin and chrysoeriol with sugar substitution at position 7 or 7, 4'; aurone and flavanone compounds (Harborne, 1963).

From the flavonoid profile of the investigated species; the ability of *K. aegyptiaca* for synthesizing the 6 and 8-hydroyflavone compounds is an advanced step from *Linaria* which can produce 6-hydroyflavone compounds only, while the lacking of such compounds from genus *Antirrhinum* let us assumed that it is less advanced species; that conclusion is in agreement with Mourad et al. (2015) who mentioned that according the floral characters the evolution line inside tribe Antirrhineae starts from *Antirrhinum* (less advanced) to *Kickxia* (more advanced). The present study suggests that *K. aegyptiaca* is the most advanced species in tribe Antirrhineae and chemically is closely related to *Veronica* species and more investigations needed to include the rest *Kickxia* species.

4. Conclusion

In conclusion, two flavonoids; isoscutellarein 8, 4′- di-OCH_3 7-*O*-rutinoside -4‴-acetate (**1**) and isoscutellarein 8, 4′- di-OCH_3-7-*O*-rutinoside; isopectolinarin (**2**) were isolated for the first time from nature along with five known flavonoids from *K. aegyptiaca*. Furthermore, the cytotoxic evaluation revealed that pectolinarigenin (**4**) possessed the highest cytotoxic activity on MCF-7 with inhibition 91.1% while, ladenine (**3**) and pectolinarin (**5**) showed moderate inhibition for MCF-7 cell line as 65.8% and 58.8%, respectively. Furthermore, the identified flavonoids were found to have high chemosystematic significance confirming that *K. aegyptiaca* is the most advanced species in tribe Antirrhineae and chemically it closely related to *Veronica* species.

Authors' contributions

MMMar and SRH created the point and carried out the design. MMF, SRH, MMMar and MES collected the plant material and worked on laboratorial work. MAS and MMMou assisted in carrying out the laboratory work. MMMar formatted and submitted the manuscript. All the authors analyzed the data, wrote, read and revised the manuscript and approved the submission.

Ethical statement

N/A.

Declaration of competing interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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