Bulletin of Faculty of Pharmacy, Cairo University (2011) **49**, 1–5

Cairo University

Bulletin of Faculty of Pharmacy, Cairo University

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ORIGINAL ARTICLE

Phenolics from *Kalanchoe marmorata* Baker, Family Crassulaceae

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Received 26 May 2011; accepted 21 June 2011
Available online 26 August 2011

KEYWORDS

Crassulaceae;
Flavonoids;
Kalanchoe marmorata;
Phenolics;
Phenolic acids

Abstract In search of plants rich in phenolics in Egypt, *Kalanchoe marmorata* Baker was subjected to phytochemical study. The preliminary phytochemical screening revealed its richness in phenolics. Fractionation of the lyophilized aqueous extract of the leaves of *K. marmorata* by different organic solvents successively resulted in the isolation and purification of five compounds from the ethyl acetate soluble fraction. These compounds namely; **E1** isorhamnetin-3-*O*-α-L-$^{1}C_{4}$-rhamnopyranoside; **E2** quercitin; **E3** 4′-methoxy-myricetin-3-*O*-α-L-$^{1}C_{4}$-rhamnopyranoside; **E4** Quercitin-3-*O*-β-D-$^{4}C_{1}$-glucopyranoside and **E5** protocatechuic-4′-*O*-β-D-$^{4}C_{1}$-glucopyranoside, were identified by analysis of their spectral data including ^{1}H NMR and ^{13}C NMR.

1. Introduction

Kalanchoe, a genus of approximately one hundred species, is native to tropical Africa but has been naturalized throughout the tropics.[1,2] Other names of genus *Kalanchoe* (family Crassulaceae) are *Bryophyllum* and *Cotelydon*.[3] The most cited in literature are *K. pinnata* Lam., *K. brasiliensis* Larranaga, *K. diagremontiona* R. Hamet, *K. spathulata* DC., *K. gracilis* Hance, *K. streptantha* Baker, *K. blossfeldiana* Poelln., and *K. tubiflora* Raym. Hamet.[4] The genus has attracted great interest so much so that it has been the subject of numerous chemical studies.

In traditional medicine, plants of genus *Kalanchoe* were used for the treatment of periodontal disease, cracking lips in children, bruises, wounds, boils, arthritis and gastric ulcers.[5,6] Other traditional uses for this species included ear infection, dysentery[7], fever, coughs[8], cholera, urinary diseases, whitlow[9] and relieving headaches.[7]

A literature survey revealed that the different extracts of various *Kalanchoe* species had been proved to possess antimicrobial[7], antiulcer[10], analgesic[11], antihyperglycemic[12], cardiovascular[13] and anti-inflammatory effects.[10] Moreover, the immunomodulatory[5,14] and hepatoprotective activities[15] were reported of *K. pinnata*. Different chemical constituents had

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Peer review under responsibility of Faculty of Pharmacy, Cairo University.
doi:10.1016/j.bfopcu.2011.07.001

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been isolated from this genus including flavonoids[9], sterols[16], anthocyanins[17], coumarins[18] and bufadienolides.[8]

Kalanchoe marmorata Baker is known as the Penwiper plant. The other name for this succulent plant is *Kalanchoe grandiflora* Rich. and it is indigenous to Ethiopia, Eritrea and Sudan.[4] *K. marmorata* Baker is commonly cultivated and used as an ornamental plant in Egypt was selected to carry out this phytochemical study. Preliminary phytochemical screening of *K. marmorata* Baker revealed that the plant is rich in phenolic compounds. This encouraged us to continue our research on phenolics from plants in Egypt. To our knowledge, no literature regarding the phytochemical composition or the biological activity of *K. marmorata* Baker could be traced (Fig. 1).

2. Experimental

2.1. Materials and methods

2.1.1. Plant material

Fresh samples of *K. marmorata* Baker were purchased from Shebeen el-Kanater/Elkalubya, Egypt in June 2009. The plants were kindly identified by Mrs. Terese Labib, the Plant taxonomist of El-Orman Botanical Garden in Giza, Cairo. A voucher specimen of the plant was deposited in the herbarium at the Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University.

2.1.2. Instruments and materials for phytochemical investigation

NMR: ^{1}H and ^{13}C NMR spectra were obtained on a Joel ECA 500 spectrometer. ^{1}H chemical shifts were measured relative to TMS scale by adding 39.5 and ^{13}C, were measured at 100 MHz, relative to DMSO-d_6 and converted to TMS width = 6000 Hz for ^{1}H and 22,000 Hz for ^{13}C. UV: UV/V Shimadzu spectrophotometer was used for the detection of flavonoids and the effect of different shift reagents. Paper chromatography (PC) analysis was carried out on Whatmann No. 1 paper, using solvent systems: (1) H_2O; (2) 6% HOAc; (3) BAW (*n*-BuOH–HOAc–H_2O, 4:1:5, upper layer) and (4) *n*-Butanol saturated with water. Solvents (2) and (3) were used for preparative PC (PPC) on Whatmann No. 3 MM. Solvents methanol, water and acetic acid of HPLC grade were purchased from Merck Ltd. (Mumbai, India). Other chemicals and reagents used were of analytical grade.

2.1.3. Extraction, isolation and purification

Fresh green leaves of the plant (2.5 kg) were sliced and homogenized in a mechanical blender with distilled water. The homogenized extract was then filtered through muslin and lyophilized to yield a dark brown residue of the water extract (93.8 g). The lyophilized water extract was dissolved in the least amount of water then partitioned using dichloromethane (2 L), ethyl acetate (2 L), *n*-butanol (700 ml) to afford 0.7 g of dichloromethane fraction, 2.1 g of ethyl acetate fraction and 1.9 g of *n*-butanol fraction.

Preparative paper chromatographic analysis of the ethyl acetate fraction (2.1 g) was carried out using Whatmann No. 3 MM paper and BAW as a solvent system. The developed chromatogram revealed 7 bands (A-G). The bands were separately eluted with methanol (HPLC grade) and the eluates were investigated by PC (BAW and 6% acetic acid). Repeated PPC of the fractions revealing the major spots (whatman No. 3 MM, 6% acetic acid) was performed. Five pure compounds were successfully isolated; compounds **E1** (yellow powder, 20 mg), **E2** (yellow powder, 10 mg), **E3** (yellow powder, 30 mg), **E4** (yellow powder, 50 mg) and **E5** (yellow powder, 10 mg). The structures of the isolated compounds were established on the basis of physicochemical data, UV spectral data with different shift reagents, ^{1}H NMR and ^{13}C NMR, as well as comparison with published data.

3. Results

*Compound **E1**:* Pale yellow amorphous powder (20 mg), which appeared as a dark purple spot on PC under UV light and turned yellow upon exposure to ammonia vapors and then turned dirty green after spraying with 1% methanolic ferric chloride solution. R_f values: 79.5 (BAW) and 44 (6% acetic acid). UV λ max: MeOH (257, 351), MeOH/NaOAc (272, 380), MeOH/NaOAc/H_3BO_3 (261, 370), MeOH/$AlCl_3$ (272, 310, 364), MeOH/$AlCl_3$/HCl (298, 348). ^{1}H NMR data: δ ppm 0.7 (3H, *d*, J = 6.0 Hz, H-6″[CH_3]), 3.1–3.7 (*m*, sugar protons), 3.9 (3H, *s*, OCH_3), 5.2 (1H, *d*, J = 1.5 Hz rhamnose, H-1″), 6.12 (1H, *d*, J = 2.5 Hz, H-6), 6.3 (1H, *d*, J = 2.5 Hz, H-8), 6.8 (1H, *d*, J = 7.8 Hz, H-5′), 7.25 (1H, *d*, J = 2 Hz, H-2′), 7.21 (1H, *dd*, J = 2, 7.8 Hz, H-6′). ^{13}C NMR data: δ ppm 157 (C-2), 134.61 (C-3), 178 (C-4), 161 (C-5), 102.3 (C-6), 165.9 (C-7), 94.33 (C-8), 157.5 (C-9), 104.1 (C-10), 121.1 (C-1′), 116.11 (C-2′), 149.14 (C-3′), 145.82 (C-4′), 116 (C-5′), 121.58 (C-6′), 99.54(C-1″), 70.58 (C-2″), 70.88 (C-3″), 71.7 (C-4″), 70.88 (C-5″), 18 (C-6″), 56.3 (OCH_3).

*Compound **E2**:* Amorphous light yellow powder (10 mg), which appeared as a yellow spot on PC under UV light. R_f values: 75.2 (BAW) and 1.07 (6% acetic acid). UV λ max: MeOH (255, 367), MeOH/NaOAc (274, 394), MeOH/NaOAc/H_3BO_3 (263, 344, 387), MeOH/$AlCl_3$ (240, 314, 362), MeOH/$AlCl_3$/HCl (252, 348). ^{1}H NMR data: δ ppm 6.1 (1H, *d*, J = 2.5, H-6), 6.37 (1H, *d*, J = 2.5, H-8), 6.83 (1H, *d*, J = 6.6 Hz, H-5′), 7.66 (1H, *dd*, J = 2.1, 6.6 Hz, H-6′), 7.48 (1H, *d*, J = 2.1, H-2′).

*Compound **E3**:* Pale yellow amorphous powder (30 mg), which appeared as a dark purple spot on PC under UV light, that changed yellow upon exposure to ammonia vapors then dirty green after spraying with 1% methanolic ferric chloride reagent. R_f values: 68.8 (BAW) and 31.18 (6% acetic acid). UV λ max: MeOH (260, 357), MeOH/NaOAc (269, 337), MeOH/NaOAc/H_3BO_3 (261, 376), MeOH/$AlCl_3$ (314, 363), MeOH/$AlCl_3$/HCl (260, 350). ^{1}H NMR data: δ ppm 0.79 (3H, *d*, J = 6.0 Hz, H-6″[CH_3]), 3.1–3.7 (*m*, sugar protons), 3.9 (3H, *s*, OCH_3), 5.1 (1H, *d*, J = 1.6 Hz rhamnose, H-1″), 6.1 (1H, *d*, J = 2.5 Hz, H-6), 6.3 (1H, *d*, J = 2.5 Hz, H-8), 6.8 (2H, *s*, H-2′,6′).

*Compound **E4**:* Pale yellow amorphous powder (50 mg), which appeared as a dark purple spot on PC under UV light, that changed yellow upon exposure to ammonia vapors then dirty green after spraying with 1% methanolic ferric chloride reagent. R_f values: 70.9 (BAW) and 19.3 (6% Acetic acid). UV λ max: MeOH (257, 358), MeOH/NaOAc (273, 399), MeOH/NaOAc/H_3BO_3 (262, 379), MeOH/$AlCl_3$ (273, 309, 364), MeOH/$AlCl_3$/HCl (259, 355). ^{1}H NMR data: δ ppm 3.1–3.7 (*m*, sugar protons), 5.4 (1H, *d*, J = 7.5 Hz glucose

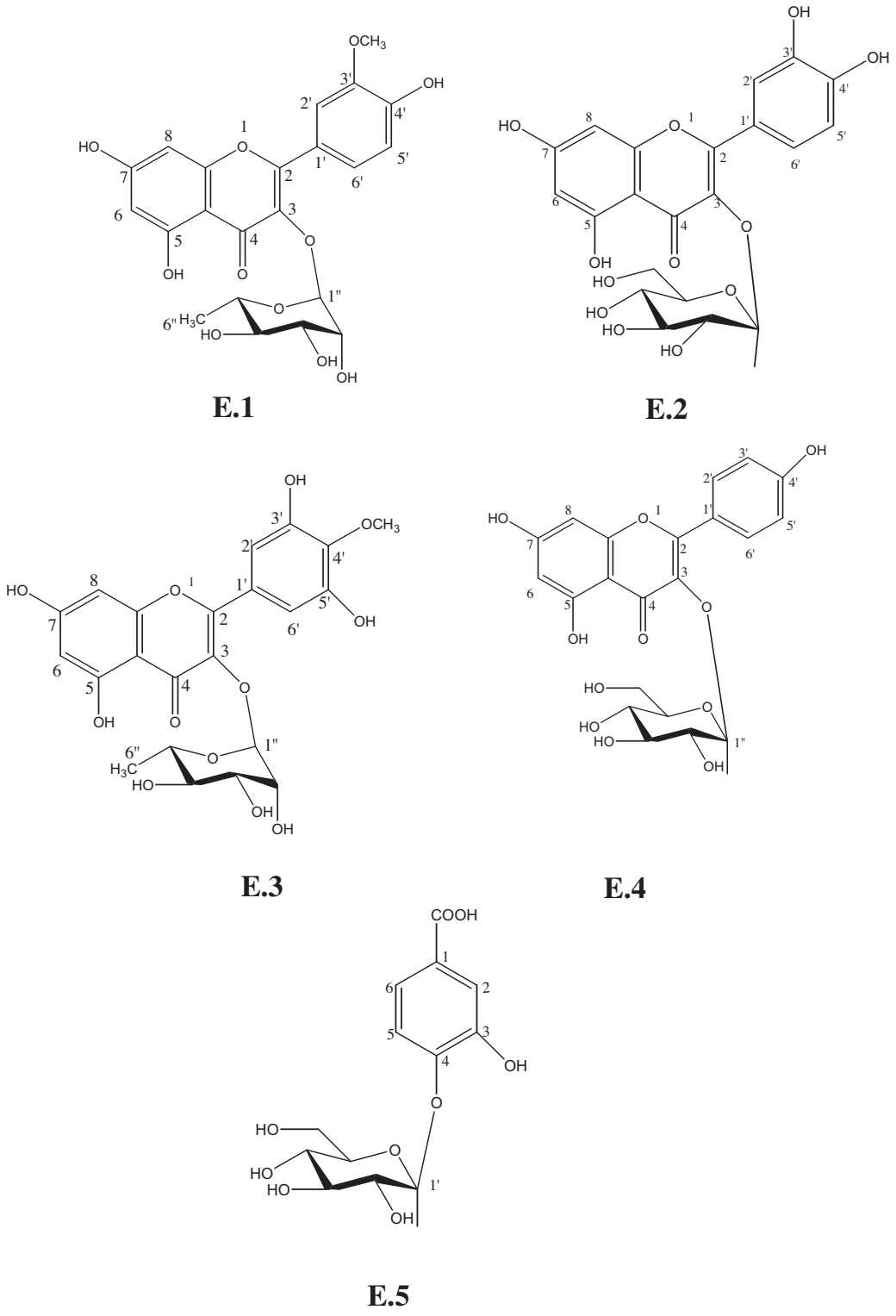

Figure 1 Structures of isolated phenolics from *Kalanchoe marmorata* Baker.

H-1″), 6.1 (1H, *d*, *J* = 2.5 Hz, H-6), 6.3 (1H, *d*, *J* = 2.5 Hz, H-8), 6.8 (1H, *d*, *J* = 6.6 Hz, H-5′), 7.54 (1H, *dd*, *J* = 2.1, 6.6 Hz, H-6′) 7.45 (1H, *d*, *J* = 2.1, H-2′). ^{13}C NMR data: δ ppm 156.7 (C-2), 133.7 (C-3), 177.9 (C-4), 161.7 (C-5), 99.1 (C-6), 164.6 (C-7), 94 (C-8), 156.8 (C-9), 104.4 (C-10), 121.6 (C-1′), 116.6 (C-2′), 145.3 (C-3′), 148.9 (C-4′), 115.7 (C-5′), 122.1 (C-6′), 101.3 (C-1″), 74.5 (C-2″), 78 (C-3″), 70.3 (C-4″), 76.9 (C-5″), 61.4 (C-6″).

Compound ***E5****:* Pale yellow amorphous powder (10 mg), which appeared as a violet spot on PC under short wavelength UV light, then changed into bluish black after spraying with 1% methanolic ferric chloride. R_f values: 73.11 (BAW) and

48.38 (6% acetic acid). UV λ max: MeOH (209). ^{1}H NMR data: δ ppm 3.1–3.7 (*m*, sugar protons), 3.9 (3H, *s*, OCH_3), 5.5 (1H, *d*, $J = 7.6$, glucose H-1′), 7.05 (1H, *d*, $J = 8.1$ Hz H-5), 7.4 (1H, *d*, $J = 2.1$ Hz, H-2), 7.7 (1H, *dd*, $J = 2.1$, 8.1 Hz H-6).

4. Discussion

4.1. Identification of the isolated compounds

*Compound **E1**:* Exhibited UV spectrum, with two bands at λ_{max} MeOH 351 nm (band I) and 257 nm (band II) which indicated the flavonol nucleus.[19] A bathochromic shift (15 nm) had occurred on the addition of sodium acetate to indicate that the hydroxyl group at the 7-position is free. An observable bathochromic shift (19 nm) in band II indicating the presence of free ortho dihydroxy at band II after the addition of H_3BO_3, confirming the presence of ortho hydroxyl groups at positions 3′ and 4′. On addition of aluminium chloride, bathochromic shifts (15 nm) in band I and (13 nm) in band II were obtained and indicated the presence of a free hydroxyl group at C-5 and free ortho dihydroxy groups at positions 3′ and 4′. The ^{1}H NMR spectrum supported the presence of 3-*o*-substitution of quercetin due to the absence of appreciable shifts on the aromatic proton resonance on ring A which showed signals at δ ppm 6.12 and 6.3 assigned for H-6 and H-8 respectively. The identity of quercetin was supported by the presence of signals at δ ppm 6.8 (1H, *d*, $J = 7.8$ Hz, H-5′), 7.25 (1H, *d*, $J = 2$ Hz, H-2′) and 7.21 (1H, *dd*, $J = 2$, 7.8 Hz, H-6′). Moreover, the anomeric proton of rhamnose displayed a signal at δ ppm 5.2 (1H, *d*, $J = 2.5$ Hz, H-1″). Other signals of sugar protons appeared at δ ppm 3.1–3.7 (*m*, sugar) and δ ppm 0.7 (3H, *d*, $J = 6$ Hz, CH_3 of rhamnose. The singlet signal appearing at δ ppm 3.9 indicated the presence of a methoxy group which favorably might be present at 3′ position. Further confirmation of the identity of compound **E1** as isorhamnetin-3-*O*-α-L-^{1}C$_4$-rhamnopyranoside was available through ^{13}C NMR spectroscopic analysis which revealed the presence of 22 distinct signals. The presence of rhamnose was confirmed by the appearance of signal at δ ppm 18 indicating the presence of the methyl group of rhamnose. Also the presence of signal at δ ppm 56.3 indicated the presence of a methoxy group. The spectral data of compound **E1** were found in accordance with those reported for isorhamnetin-3-*O*-α-L-^{1}C$_4$-rhamnopyranoside.[20]

*Compound **E2**:* Exhibited a UV spectrum, with two bands at λ_{max} MeOH 367 nm (band I) and 255 nm (band II) which indicated a flavonol nucleus.[19] A bathochromic shift (19 nm) was obtained on addition of sodium acetate to indicate that the hydroxyl group is at 7-position. Upon addition of boric acid, a bathochromic shift occurs 13 and 20 nm in bands II and I respectively, indicating the presence of a hydroxyl group at position 5 and the presence of *ortho* hydroxyl groups at positions 3′ and 4′. ^{1}H NMR analysis showed signals at δ ppm 6.1 (1H, *d*, $J = 2.5$ H-6) and 6.37 (1H, *d*, $J = 2.5$, H-8). The identity of quercetin was supported by the presence of signals at δ ppm 7.66 (1H, *d*, $J = 2.1$ H-6′), 7.48 (1H, *dd*, $J = 2.1$, 6.6, H-2′) and 6.83 (1H, *d*, $J = 6.6$ Hz, H-5′). The compound was identified as quercetin compared with reported spectral data in literature.[21]

*Compound **E3**:* Exhibited a UV spectrum, which showed two bands at λ_{max} MeOH (357 nm band I and 260 nm band II) which indicated the flavonol nucleus.[19] It showed a bathochromic shift (9 nm) with sodium acetate in band II to prove that the hydroxyl group at 7 position is free. A bathochromic shift (19 nm) in band I was observed upon addition of boric acid. This indicated the presence of *ortho* hydroxyl groups at positions 3′ and 4′. The bathochromic shift (54 nm) obtained upon addition of aluminium chloride, followed by a hypsochromic shift upon addition of HCl, indicated the presence of a free hydroxyl group at C-5. ^{1}H NMR spectrum of compound **E3** showed the presence of aromatic proton signals at δ ppm 6.1 and 6.30 indicating the presence of protons at 6 and 8 positions respectively. The presence of a singlet signal appearing at δ ppm 6.84 indicated the protons at H-2′ and H-6′ confirming the structure of myricitin. The anomeric proton appearing at δ ppm 5.1 (*d*, $J = 1.6$ H-1″) together with the presence of a signal at δ ppm 0.79 (3H, *d*, $J = 6$) indicated the methyl of rhamnose. The appearance of a signal at δ ppm 3.9 (3H, *s*) confirmed the presence of a methoxy group. The compound was thus identified as 4′-methoxy-myricetin-3-*O*-α-L-^{1}C$_4$-rhamnopyranoside as compared with spectral data in literature.[22]

*Compound **E4**:* Exhibited a UV spectrum with two bands at λ_{max} MeOH (358 nm band I and 257 nm band II) which indicated a flavonol nucleus.[19] The bathochromic shift (16 nm) produced in band II on addition of sodium acetate proved that the hydroxyl group at position 7 is free. The chemical shift (16 nm) obtained after the addition of aluminium chloride indicated the presence of a free hydroxyl group at C-5. ^{1}H NMR spectral analysis confirmed the presence of 3-*O*-substitution of quercetin. It showed aromatic proton signals at δ ppm 6.1 and 6.30 indicating protons at position 6 and 8 position respectively. Deshielded aromatic protons at δ ppm 6.8, 7.45 and 7.54 indicated protons at 5′, 2′, 6′ respectively. A signal appearing at δ ppm 5.4 (1H, *d*, $J = 7.5$ Hz H-1″) represented the anomeric proton of glucose. The compound was identified as quercetin-3-*O*-*B*-D-^{4}C$_1$-glucopyranoside.[23]

*Compound **E5**:* The UV absorbance spectrum revealed one intense peak at λ_{max} MeOH (209 nm). ^{1}H NMR spectrum showed the presence of an aromatic proton signal at δ ppm 7.05 (1H, *d*, $J = 8.1$ Hz) indicating an *ortho* substituted proton H-5. The second proton appearing at δ ppm 7.4 (1H, *d*, $J = 2.1$ Hz) indicated a *meta* substituted proton H-2. The third proton appeared as a doublet of doublet at δ ppm 7.7 (1H, *dd*, $J = 2.1$, 8.1 Hz) indicating ortho and meta substituted protons i.e. trisubstituted benzene ring. The presence of an anomeric proton at δ ppm 5.5 (1H, *d*, $J = 7.6$ Hz) indicated a glucosidic linkage. The compound was identified as protocatechuic-4′-*O*-β-D-^{4}C$_1$-glucopyranoside.[24]

5. Conclusion

From the ethyl acetate soluble fraction of the aqueous extract of the leaf of *K. marmorata* Baker, four flavonols and a phenolic acid glucoside were isolated and identified as **E1** isorhamnetin-3-*O*-α-L-^{1}C$_4$-rhamnopyranoside; **E2** quercetin; **E3** 4′-methoxy-myricetin-3-*O*-α-L-^{1}C$_4$-rhamnopyranoside; **E4** quercetin-3-*O*-β-D-^{4}C$_1$-glucopyranoside and **E5** protocatechuic-4′-*O*-β-D-^{4}C$_1$-glucopyranoside. Both **E2** quercetin- and **E4** quercetin-3-*O*-β-D-^{4}C$_1$-glucopyranoside were previously isolated from the flowers of *K. blossfeldiana*.[17] **E1** isorhamnetin-3-*O*-α-L-^{1}C$_4$-rhamnopyranoside, **E3** 4′-methoxy-myricetin-3-*O*-α-L-^{1}C$_4$-rhamnopyranoside and **E5** protocatechuic-4′-*O*-β-D-^{4}C$_1$-glucopyranoside were isolated for the first time in this genus.

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