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Phytochemistry 64 (2003) 883–889
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Polyoxygenated flavonoids from *Eugenia edulis*

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Received 24 February 2003; accepted 24 June 2003

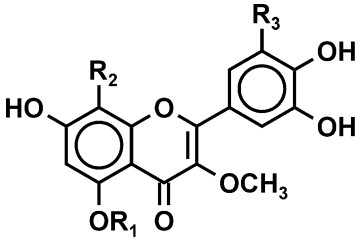
Abstract

Leaves of *Eugenia edulis* contain the new polyoxygenated flavonoid derivatives, gossypetin-3,8-dimethyl ether-5-*O*-β-glucoside; gossypetin-3,5-dimethyl ether, and myricetin-3,5,3′-trimethyl ether. In addition, ten known polyphenolics were also isolated and identified. All structures were established on the basis of chemical and spectral evidence, including ESI-MS and ^{13}C NMR.

Keywords: *Eugenia edulis*; Myrtaceae; Polyoxygenated flavonoids; gossypetin-3,8-dimethyl ether-5-*O*-β-glucoside; gossypetin-3,5-dimethyl ether; myricetin-3,5,3′-trimethyl ether; ESI-MS; NMR

1. Introduction

Eugenia edulis Vell (syn. *E. cauliflora* D. C.; *Myrciaria edulis* Skeel; *Myrtus cauliflora* Mart and *Myrciaria jaboticaba* Berg), known in english as ''Jaboticaba'' is an evergreen tree of Brazilian origin. However, it is cultivated, mostly in the tropics and subtropics. The species is a slow growing very branched tree, up to 12 m height (Bailey, 1933), which produces cherry-like edible berries. Although, different parts of several *Eugenia* species were proved to provide extracts which are used in traditional medicine as anti-microbial and anti-inflammatory agents (Djipa et al., 2000; Slowing et al., 1994a) and known to contain appreciable concentrations of polyphenolics of the ellagitannin and flavonoid types (Lee et al., 1997; Slowing et al., 1994b) yet, the fruit were the only part of *E. edulis* (Myrtaceae) which have been previously investigated for their flavonoids (Travisan et al., 1972). During preliminary phytochemical analysis, phenolics were shown to be the major leaf constituents of this plant, this prompted us to investigate them in more detail. The present paper describes the isolation and structural elucidation, from the aqueous alcoholic (75%) leaf extract of *E. edulis*, of three new polyoxygenated derivatives, namely, gossypetin-3,8-dimethyl ether-5-*O*-β-glucoside (**4**); gossypetin-3,5-dimethyl ether (**10**), and myricetin-3,5,3′-trimethyl ether (**11**). Ten known polyphenolics (**1**–**3**, **5**–**9**, **12** and **13**) also, were isolated and characterized from the same extract.



Compound 4: R_1=Glc, R_2=OCH_3, R_3=H
Compound 10: R_1=CH_3, R_2=OH, R_3=H
Compound 11: R_1=CH_3, R_2=H, R_3=OCH_3

2. Results and discussion

The concentrated 75% aqueous ethanol extract from a homogenate of the dried leaves of *E. edulis* was fractionated by column chromatography over polyamide S6, using water/methanol mixtures of decreasing polarities to yield nine different fractions. Repeated Sepadex

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0031-9422/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00437-0

LH-20 column fractionation of the 40 and 80% aqueous methanol polyamide column fractions afforded pure samples of (**4**) from the former fraction and of (**10** and **11**) from the latter. The known compounds (**1-3**, **5–9**, **12** and **13**) were isolated and purified, from the remaining polyamide column fractions by standard methods (column and preparative paper chromatography). They gave chromatographic, UV absorption, hydrolytic, ESI-MS, ^{1}H and ^{13}C NMR data identical with those reported for gallic acid (**1**), (Nawwar and Hussein, 1994); protocatechuic acid (**2**), (Bremser et al., 1980); *epi*-gallocatechin-3-*O*-gallate (**3**), (Shen et al., 1993); quercetin-3-*O*-β-galactoside (**5**), (Nawwar et al., 1984a); quercetin-3-*O*-β-(6″-galloylglucoside) (**6**), (Merkham, 1978); kaempferol-3-*O*-α-arabinofuranoside (**7**), (Harborne and Williams, 1975); quercetin-3-*O*-α-arabinofuranoside (**8**), (Agrawal, 1989); myricetin-3-*O*-α-rhamnoside (**9**), (Marco, Babera, Sanz and Sanchez-Parareda, 1985); kaempferol (**12**) and quercetin (**13**), (Nawwar et al., 1984b).

The new compound **4** was found to possess flavonoid nature (red colour with Mg/HCl) and exhibited chromatographic properties (a fluorescent light blue spot, R_f values: Table 1) and UV spectral maxima, in methanol and after addition of diagnostic shift reagents specific for flavonoids (Harborne and Williams, 1975; Mabry et al., 1970), (Table 1), comparable to those reported for derivatives of 3,5-di-*O*-substituted-7-hydroxyflavonols [small bathochromic shift (21 nm) of band I, on addition of $AlCl_3$, which disappeared on addition of HCl]. Furthermore, the UV spectral data suggested the presence of a free 7-OH and a di-*ortho*-hydroxy group(s) in the molecule of **4** (positive shifts of band II with CH_3COONa and of band I with $CH_3COONa + H_3BO_3$). The ESI-MS spectrum of **4**, in negative mode, exhibited a molecular ion $[M–H]^-$ at $m/z = 507$, corresponding to a molecular mass of 508 and a molecular formula of $C_{23}H_{26}O_{13}$. The spectrum also exhibited a fragment ion at m/z 345. This ion is obviously, attributable to the aglycone moiety **4a** of component **4**. The above obtained MS results indicated also that **4a** is glycosidated by a hexose moiety to produce the parent compound **4** (molecular mass = 508). MS/MS analysis of the fragment ion 345 exhibited, in its spectrum, a daughter ion at $m/z = 330$. The latter, in turn, showed, on MS/MS analysis a daughter ion at $m/z = 315$. These MS/MS data confirmed the sequential losses of two methyl group from the aglycone moiety **4a** thus suggesting, together with the above given analytical data, that the parent component **4** is, most probably, a dimethoxy-tetrahydroxyflavonol hexoside.

Complete acid hydrolysis of **4** (2 N aqueous methanolic (1:1) HCl, 7 h, 100 °C). afforded glucose (comparative paper chromatography), together with the aglycone **4a**. This aglycone was also, released from **4** on β-glucosidase enzymatic hydrolysis, by being incubated with the enzyme, in an acetate buffer of pH 5.2, for 24 h, at 37 °C. It appeared on paper chromatograms, under UV light as a discrete spot of a dark greenish purple colour (R_f-values, Table 1), thus reflecting a change in the proposed 3,5-di-*O*-substitution character of the parent component **4**. The aglycone **4a** also, differed markedly, in its UV spectral maxima, (Table 1) from the parent compound **4** and exhibited, in negative ESI-MS, a molecular ion at $m/z = 345$, corresponding to a molecular mass of 346. On hydrolytic cleavage (Vogel, 2001), by being refluxed with concentrated HI and phenol (0.5, 145 °C), **4a** afforded a detherification product **4b,** which appeared, on paper chromatograms, as black spot under UV light and which was not affected on fuming with ammonia vapour, but turned green in V light. It was found identical with respect to its R_f-values and UV spectral data (Table 1) with authentic 3,5,7,8,3′,4′-hexahydroxyflavone, gossypetin (Nawwar and Buddrus, 1981). Consequently, **4a** should possesses a gossypetin-dimethyl ether structure.

For final elucidation of the structure of **4a**, it was therefore, subjected to ^{1}H NMR spectroscopic analysis. The presence, in this spectrum (DMSO-d_6, room temperature), of two OCH_3 functions followed from the

Table 1
R_f-values and UV spectral data of compounds (**4**, **10** and **11**) and their hydrolysis and de-etherification products

Compound	R_f-values (x 100)			UV spectral data $\lambda_{max\ (nm)}$					
	H_2O	AcOH-6	BAW	MeOH (a)	(a) + NaOAc (b)	(b) + H_3BO_3	(a) + $AlCl_3$ (c)	(c) + HCl	MeONa
4	23	37	38	255, 269, 354	274, 317, 372	263, 373	262, 296, 375	253, 268[a], 352	272, 395
4a	00	00	58	260, 271, 358	279, 312, 400	266, 378	274, 304[a], 363, 428	273, 359, 405	276, 345, 414
Gossypetin	00	00	48	260, 278, 382	280, 365[b]	273, 282, 366[b]	190, 325, 401, 488	272, 313, 372, 444	252, 285, 364[b]
10	00	08	51	255, 272, 351	280, 319, 367	264, 367	238, 275, 335, 379	238, 255, 272, 350	271, 396
11	00	10	65	252, 265, 347	271, 322, 361	254, 297, 368	256, 374	252, 260[a], 305, 348	263, 317, 397
Myricetin	00	11	62	254, 272[a], 301[a], 374	269, 335[a]	258, 304[a], 392	271, 316[a], 450	266, 275[a], 360[a]	262[a], 285[a], 322, 423[a]

[a] Inflection.
[b] Decomposition.

two proton singlets recognized at δ ppm 3.74 and 3.70, assignable to the protons of these two functions. All remaining resonances, in this spectrum were due to protons attached to sp^2 carbons and appeared at δ ppm 6.05 (s); 6.88 (*d*, $J=8.0$ Hz); 7.50 (*dd*, $J=8.0$ and 2.5 Hz); 7.75 (*d*, $J=2.5$ Hz), a pattern which was found similar to that reported for the resonances of H-6, H-5′, H-6′ and H-2′ of gossypetin 3,8-dimethyl ether (Horie et al. 1987). The EI-MS spectrum of **4a** exhibited ion peaks at *m*/*z* (rel. int.) 346 $[M]^+$ (76) indicating a gossypetin aglycone with two methoxyl substituents. It showed also ion peaks at *m*/*z* 331 $[M\text{-}15]^+$ (100), 345 $[M\text{-}1]^+$ (20) and the retro Diels–Alder fission gave rise to ion peaks at *m*/*z* 182 $[A_1]^+$ (64), 167 $[A_1\text{-}15]^+$ (33), 139 $[A_1\text{-}43]^+$ (28), 137 $[B2]^+$ (36), 109 $[B_2\text{-}28]^+$ (22), thus proving the presence of a 8-OCH_3 function in ring A and two hydroxyl functions in ring B of the flavonol aglycone **4a.** These EI-MS data together with the presence of a recognizable $[M\text{-}1]^+$ ion proved the structure **4a** to be gossypetin-3,8-dimethyl ether (Mabry and Markham, 1975), a structure which is also, in consistent with the chromatographic properties and UV absorption maxima exhibited by this aglycone (Table 1). These data led to the conclusion that the parent compound **4** should possess a gossypetin 3,8-dimethylether-5-β-glucoside structure.

The ^1H NMR spectrum of **4** was found to be quite similar to that of the aglycone **4a**. However, a distinction could be made by the recognition of a proton doublet at δ ppm 4.35 of coupling constant = 8.0 HZ, assignable to a β-anomeric proton of a glucose moiety adopting a $^4C_1$ conformation. The remaining glucose proton resonances in this spectrum, revealed themselves as overlapping multiplets in the region from δ ppm 3.15–3.37. The chemical shift value of the anomeric glucose proton resonance ensure that this moiety is not sited at the C-3 position of the aglycone moiety, otherwise the location of this resonance should be shifted more downfield (≈5.5 ppm), (Mabry et al., 1970).

For precise determination of the site of attachment of the two OCH_3 and glucose functions and for final confirmation of the identity of component **4** as well, ^{13}C NMR analysis was therefore, performed. The recorded spectrum, revealed a methoxyl carbon resonance at δ ppm 59.7, a location which is typical for the carbon resonance of an OCH_3 located at the flavonol position number 3 (Horie et al., 1998). Methyl etherification at C-3 of the aglycone moiety was further proved by the characteristic downfield shift of the resonance of this carbon to δ ppm 139.4 and the accompanying upfield chemical shift of the carbonyl carbon C-4 resonance to δ ppm 173.1 [all in comparison with the chemical shifts of the corresponding carbon resonances in the spectrum of the flavonol gossypetin (Nawwar and Buddrus, 1981)]. The spectrum revealed also a second methoxyl carbon resonance at δ ppm 59.9 accompanied by a recognizable downfield shift of the aglycone C-8 carbon to δ ppm 129.0, thus confirming that the second OCH_3 function is located at the C-8 carbon of the aglycone. In addition, the spectrum revealed an upfield location of the resonance of the C-5 carbon (δ ppm 151.2), and a downfield location of the resonances of the C-6 and C-10 carbons (δ ppm 100.3 and 108.95, respectively) with respect to the locations of the corresponding carbon resonances in the spectrum of the free flavonol gossypetin. These chemical shifts are best interpreted in terms of a 5-*O*-glucosidation of the aglycone **4a** to produce the parent component **4**. Other carbon resonances, In this spectrum possessed chemical shifts (Table 2) which also, fitted well with the structure of component **4** as gossypetin-3,8-dimethyl ether-5-*O*-β-$^4C_1$-glucopyranoside. This flavonol glucoside is a new natural product which is reported here to occur in nature for the first time.

Compound **10** exhibited chromatographic properties and UV spectral data (Table 1) of flavonols which might bear a 3,5-di-methyl ether functions (Mabry et al., 1970). The ESI-MS, in negative mode of **10** was in accordance with this suggestion and revealed a molecular ion [M–H]− at *m*/*z* = 345, corresponding to a molecular mass of 346 and a molecular formula of $C_{17}H_{14}O_8$. The MS/MS measurement, ensured absence of any substitution other than methyl etherification. This followed from the two daughter ions recognized at *m*/*z* = 329.93 and at 315.07, thus proving the sequential losses of two methyl groups. Consequently compound **10** should possess a dimethoxy-tetrahydroxyflavonol structure.

The recovery of compound **10** unchanged after drastic acid hydrolysis (methanolic 2 N HCl, 100 °C for 7 h), further confirmed the absence of hydrolysable substituents. On hydrolytic cleavage [reflux over heating mantel with HBr (40%), for 0.5 h], compound **10** yielded a detherification product **10a**, which was extracted by ethyl acetate from the reaction mixture. It appeared on paper chromatograms as black spot under UV light, which was not affected on fuming with ammonia vapour, but turned green in V light. It was found identical with respect to its R_f-values and UV spectral data (Table 1) with authentic 3,5,7,8,3′,4′-hexahydroxyflavone, gossypetin. The release of gossypetin through hydrobromic acid treatment led to the conclusion that the two ether function of the parent compound **10**, should be located at its C-3 and C-5 positions. Location at any other positions would not be affected by this reagent and needs a much stronger one (e.g. hydroiodic acid) to cause cleavage of the methyl ether functions (Harborne and Williams, 1975). The ^1H NMR data of **10** supported this view and gave a spectrum (DMSO-d_6, room temperature) which revealed two distinct methoxyl proton singlets at δ 3.71 and 3.73 ppm, together with four aromatic proton resonances at δ 6.43 (*s*), 7.6 (broad singlet), 6.89 (*d*, $J=8.0$ Hz), 7.50 ppm

Table 2
^{13}C NMR chemical shifts[a] (δ ppm) of compounds **4**, **10** and **11**

Carbon	**4**	**10**	Gossypetin	**11**[c]	Myricetin
2	151.2	151.3	146.6	152.3	147.2
3	139.4	140	135.4	140.5	136.7
4	172.3	173.1	176.1	172.7	177.9
5	151.2	152.7	152.6	161.2	161.4
6	100.3	97	98.1	96.7	99
7	156.9	152.7	152.1	163.1	165.7
8	129	126.7	122.3	95.4	94.33
9	148.2	147.8	144.8	158.5	156.6
10	109	108.1	102.7	107.8	104.3
1′	121.3	120.8	124.7	120.6	119.8
2′	116.2	116	115.5	109.6	108.01
3′	145.4	145.6	145	148.6	145.9
4′	148.2	148.5	147.7	137.5	137.5
5′	116.1	115.1	115.3	146.1	145.9
6′	122.8	122.1	120.2	104.1	108
OCH_3-3,	59.7	59.7		59.73	
OCH_3-8	59.9				
OCH_3-5		56.41		56.49[b]	
& OCH_3-3′					
1	102.9			56.40[b]	
2	74.8				
3	77.4				
4	69.3				
5	77.5				
6	60.6				

[a] NMR solvent: DMSO-d_6
[b] Assignments could be reversed.
[c] Calculated chemical shift values (δ ppm) for compound **11**: 152.22 (C-2), 140.46 (C-3), 173.90 (C-4), 161.14 (C-5), 96.90 (C-6), 165.01 (C-7), 95.93 (C-8), 159.03 (C-9), 108.97 (C-10), 120.12 (C-1′), 110.41 (C-2′), 148.60 (C-3′), 138.90 (C-4;), 145.90 (C-5′), 104.41 (C-6′).

(dd, $J = 8.0$ Hz and 2.5), assignable to the H-6, H-2′, H-5′ and H-6′, respectively. On the other hand, the EI-MS spectrum of **10** had ion peaks at m/z (rel. inten.): 346 (100%) $[M]^+$, 345 (95%) $[M–H]^+$, 328 (34%) $[M\text{-}18]^+$, 329 (32%) $[M\text{-}17]^+$, 182 (32%) $[A_1]^+$, 183 (24%) $[A_1 + H]^+$, 137 (94%) $[B_2]^+$ and 109 (21%) $[B_2\text{-}28]^+$, thus confirming the structure of **10** as 3,5-dimethoxy gossypetin, a structure which can explain the measured UV spectral data (positive shifts with NaOAc; NaOAc + H_3BO_3; no shift with $AlCl_3$ and a stable shift with NaOMe) exhibited by **10.**

The ^{13}C NMR analysis further confirmed the achieved structure of **10**. The spectrum revealed, well separated, 17 carbon resonances, the two most upfield of which at δ ppm 56.4 and 59.7 were attributed to the carbons of the methoxyl groups at the flavonol position numbers 5 and 3, respectively (Horie et al., 1998). Etherification at position 3 followed from the downfield shift of C-3 to δ ppm 140.0, while the recognizable upfield shift of the carbonyl carbon resonance to δ ppm 173.1 can obviously, be attributed to the fact that both of the flavonol positions number 3 and 5 are etherified. Assignments of the remaining chemical shifts (Table 2) was based mainly, on comparison with the ^{13}C NMR data of gossypetin (Nawwar and Buddrus, 1981) and also, by applying the methyl etherification substituent additive rules on its ^{13}C NMR data. The measured chemical shifts agreed well with the calculated values, thus finally confirming the structure of component **10** to be 7,8,3′,4′-tetrahydroxyflavone-3,5-dimethyl ether, gossypetin-3,5-dimethyl ether. This is the first reported natural occurrence of this new natural product.

Compound **11**, a faint yellow flavonoid material (red colour with Mg/HCl), which appeared on paper chromatograms as a discrete spot of white fluorescence under UV light (turned bright yellow when fumed with ammonia). The spot turned green on spraying with $FeCl_3$ and possessed the R_f-values, given in Table 1. The UV spectral properties of **11**, in methanol and after addition of the usual shift reagents (Table 1) revealed the presence of a free 7-hydroxyl group, a free di-*ortho*-hydroxyl group and absence of 3- and 5-free hydroxyl groups. This followed from the recognizable bathochromic shifts with NaOAc; NaOAc/H_3BO_3; the small bathochromic shift with $AlCl_3$, which vanishes on the addition of HCl. On negative ESI-MS/MS analysis, component **11** exhibited a molecular ion [M–H]−at m/z-359, corresponding to a molecular mass of 360 and a molecular formula of $C_{18}H_{16}O_8$. The spectrum also, revealed a daughter ion at m/z 344, thus proving the losses of a methyl group. The MS/MS spectrum of the 344 ion revealed two daughter ions at 330 and 315 to indicate the sequential losses of two additional methyl groups, thus proving that the molecule of component **11** contains 3 methyl functions. This and the above given result proved that two of the three methyl functions are located at the C-3 and C-5 positions of the flavonol molecule of component **11**. The recovery of **11** unchanged after drastic acid hydrolysis (2 N aqueous methanolic HCl, 100 °C, 7 h) further proved the absence of hydrolysable moiety in its molecule. Hydrolytic cleavage (conc. HI, 0.5 h, 145 °C) yielded a product **11a**. It appeared on paper chromatograms as a yellow spot on paper chromatograms under UV light, which turned light red on fuming with ammonia vapour. **11a** was found identical with respect to its R_f-values and UV spectral data (Table 1) with authentic 3,5,7,3′,4′;5′-hexahydroxyflavone, myricetin (Mabry et al., 1969).

The ^{1}H NMR spectrum (room temperature, DMSO-d_6), of **11** was very informative and showed three distinct methoxyl proton resonances at δ ppm 3.70, 3.80 and 3.82. In the aromatic region, the spectrum revealed also, two *meta* coupled proton doublets ($J = 2.5$ Hz) at δ ppm 6.35 and 6.48, each integrated to one proton, assignable to the H-6 and H-8 protons in the molecule of **11.** The recognizable downfield shift of the H-6 proton resonance (on comparison with the corresponding resonance in the spectrum of free myricetin) is clearly, due to the etherification of its vicinal OH-5. In this spectrum another pair of *meta* coupled ($J = 2.0$ Hz)

aromatic proton resonances were recognized at δ 7.19 and 7.20 ppm, each integrated to one proton. They were attributed to the H-2′ and H-6′ protons in an asymmetric flavonol B ring possessing a 4′,5′-dihydroxy-3′-methyl ether substitution pattern. A 2D-COSY experiment proved this assignment. Consequently, the structure of **11** must be 7,4′,5′-trihydroxyflavone-3,5,3′-trimethyl ether. For confirmation, EI-MS analysis was then performed. The spectrum showed ion peaks at *m*/*z* (rel. inten.): 360 (100%) $[M]^+$, 359 (95%) $[M–H]^+$, 342 (30%) $[M\text{-}18]^+$, 343 (22%) $[M\text{-}17]^+$, 182 (27%) $[A_1]^+$, 183 (20%) $[A_1+H]^+$, 167 (94%) $[B_2]^+$ and 139 (21%) $[B_2\text{-}28]^+$, thus proving the suggested 3,5,3′-trimethoxymyricetin structure for **11**. For final confirmation of this structure, ^{13}C NMR analysis of **11** was undertaken. Resonances in this spectrum were assigned by applying the substituent rules on the ^{13}C NMR data reported for myricetin-3′-methyl ether (Agrawal, 1989). Effect of etherification at the C-3 and C-5 positions of the flavone moiety was recognizable as it brought the resonances of the etherified carbons C-3 and C-5, as well as that of the carbonyl carbon C-4 to characteristic locations in the spectrum (δ ppm 140.5′ 161.2 and 172.7, respectively). Other chemical shifts in the aromatic region (Table 2) agreed well with the values calculated for myricetin-3,5,3′-trimethyl ether. In addition, three distinct methoxyl carbon resonances have revealed them selves, in this spectrum, at δ ppm 56.40, 56.49 and 59.73, to confirm, together with the above given data, that the structure of component **11** is 7,4′,5′-trihydroxyflavone-3,5,3′-trimethyl ether, myricetin-3,5,3′-trimethyl ether. This is a new natural product which has not been reported previously to occur in nature.

3. Experimental

^1H NMR spectra were measured at 300 MHz. ^1H chemical shifts were measured relative to TMS and ^{13}C NMR chemical shifts to DMSO-d_6 and converted to TMS scale by adding 39.5. Typical conditions: spectral width = 4000 Hz for ^1H and 19,000 Hz for ^{13}C, 32 K data points and a flip angle of 45. ESI-MS spectra were measured on SSQ Finnigan MAT 4600 quadrupole mass spectrometer (Institut für Chemie, Humboldt Universität, Berlin). EI-MS spectra were performed on SSQ Finningan MAT SSQ 7000 mass spectrometer (National Research Center, Cairo).

Paper chromatographic analysis was carried out on Whatman No. 1 paper, using solvent systems: (1) H_2O; (2) 6% HOAc; (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 1 and 3 were used for preparative paper chromatography on Whatman no. 3MM. Solvents 3 and 4 were used for sugar analysis.

3.1. Plant material

Fresh leaves of *Eugenia edulis* Vell., were collected from the Zoo garden of Cairo, Egypt, during April 2001 and authenticated by Dr. M. El-Gibali, National Research Centre (NRC), Cairo, Egypt. A voucher specimen is deposited at the NRC (voucher number is NRC-voucher: 208).

3.2. Isolation and identification

The ground dried leaves of *E. edulis* were homogenized in EtOH–H_2O (1:3) mixture. (3 kg, three extractions each with 5 l). The concentrated, filtrate of the homogenate was applied to a polyamide 6S (Riedel-De-Haen AG, Seelze Hannover, Germany) column (125×5 cm internal diameter) and eluted with water followed by H_2O–MeOH mixtures of decreasing polarities to yield 9 fractions. Compounds (**1** and **2**) were isolated, from the 20% fraction by applying repeated Sephadex LH-20 column fractionation, using ethanol (95%) for elution, which led to the successive desorption of these compounds. Crystallization, from H_2O, of the crude materials afforded pure samples of (1, 94 mg) and (2, 101 mg). Compound (**3**) was isolated, from the 30% fraction by applying repeated Sephadex LH-20 column fractionation and elution with H_2O–EtOH (50:50) mixture to yield a pure sample (**3**, 102 mg). Compound (**4**) was isolated, from the 40% fraction by applying repeated Sephadex LH-20 column fractionation and elution with H_2O–EtOH (30:70) mixture to yield a pure sample (4, 142 mg). **5** And **6** were separated from the 50% column fraction through fractionation over polyamide column and elution with a mixture of MeOH–C_6H_6–H_2O (60:38:2), followed by crystallization of the corresponding desorbed materials, from aqueous ethanol (75%) to yield crystalline (**5**, 133 mg) and (**6**, 98 mg). Compounds (**7**, 121 mg and **8**, 135 mg) were individually isolated pure from the 60% column fraction, by applying the same technique, used for the separation of **5** and **6**. Compound **9** was obtained pure (78 mg) by applying repeated Sephadex LH-20 column fractionation and elution with H_2O–EtOH (50:50). Compounds **10** (116 mg) and **11** (106 mg) were separated pure from the 80% column fraction by applying polyamide column fractionation, using ethyl acetate water saturated for elution. Both compounds **12** and **13** were separated pure (76 and 101 mg, respectively) from the 90% column fraction, by applying preparative paper chromatography, using BAW as solvent for irrigation.

3.2.1. Gossypetin-3,8-dimethyl ether-5-O-β-glucopyranoside (4)

R_f-values : UV λ_{max}^{MeOH} nm: (Table 1). ESI-MS of (**4**): negative molecular ion $[M–H]^-$: *m*/*z* = 507 and a fragment ion: *m*/*z* = 345 [M–hexoside]$^-$; MS/MS of the

345 ion: $[345\text{-}CH_3]^-$ at $m/z=330$; MS/MS of the 330 ion: $[330\text{-}CH_3]^-$ at $m/z=315$. Complete acid hydrolysis (22 mg of **4** refluxed with 10 ml aqueous methanolic, 1:1, 2 N HCl, at 100 °C, for 7 h), followed by removal of MeOH under reduced pressure and extraction by ethyl acetate, washing of the organic layer with water, filtration through anhydrous Na_2SO_4, and dryness in vacuum afforded aglycone **4a**: R_f-values: UV λ_{max}^{MeOH} nm: Table 1; ESI-MS of (**4a**): negative molecular ion $[M–H]^-$: $m/z=345$; ^1H NMR of (**4a**): δ ppm: 6.05 (s, H-6), 7.75 (*d*, $J=2.5$ Hz, H-2′), 6.88 (d, $J=8.0$ Hz, H-5′), 7.50 (dd, $J=8.0$ Hz and 2.5 Hz, H-6′), 3.74 (*s*, OMe), 3.70 (*s*, OMe); EI-MS of **4a**: *m/z* (rel. int.): 346 $[M]^+$ (76), 331 $[M\text{-}15]^+$ (100), 345 $[M\text{-}1]^+$ (20), 182 $[A_1]^+$ (64), 167 $[A_1\text{-}15]^+$ (33), 139 $[M\text{-}43]^+$ (28), 137 $[B_2]^+$ (36), 109 $[B_2\text{-}28]^+$ (22); Hydrolytic cleavage of **4a**: 11 mg were refluxed with conc. HI (3 ml) and phenol (11 mg), over a heating mantel, for 0.5 h, followed by dilution with excess of saturated $NaHSO_3$ aqueous solution and extraction of the cold reaction mixture with ethyl acetate, washing of the organic phase with bidistilled water, filtration through anhydrous Na_2SO_4 and dryness in vacuum, afforded a chromatographically pure sample (4 mg) of a dark brown sample of the detherification product **4b**: R_f-values: UV λ_{max}^{MeOH} nm: Table 1. β-Glucosidase hydrolysis of **4**: 5 mg, incubated in an acetate buffer of pH 5.2, for 24 h, at 37 °C yielded **4a**. ^1H NMR of **4**: δ ppm: 6.10 (*s*, H-6), 7.75 (*d*, $J=2.5$ Hz, H-2′), 6.92 (*d*, $J=8.0$ Hz, H-5′), 7.68 (*dd*, $J=8.0$ Hz and 2.5 Hz, H-6′), 3.70 (*s*, OMe), 3.74 (*s*, OMe), 4.35 (*d*, $J=8$ Hz, H-1″), 3.15–3.37 (overlapped sugar protons). ^{13}C NMR of **4**: Table 2.

3.2.2. Gossypetin-3,5-dimethyl ether (*10*)

R_f-values (Table 1); UV λ_{max}^{MeOH} nm: Table 1; ESI-MS negative molecular ion: $m/z=345$ $[M–H]^-$; MS/MS: of the 345 ion: $[345\text{-}CH_3]^-$; at $m/z=330$; MS/MS of the 330 ion: $[330\text{-}CH_3]^-$ at $m/z=315$; complete acid hydrolysis (11 mg of **10** refluxed with 5 ml aqueous methanolic, 1:1, 2 N HCl, at 100 °C, for 7 h): no changes. Hydrolytic cleavage of **10**: 16 mg were refluxed with conc. HBr (40%, 3 ml), over a heating mantel, for 0.5 h, followed by dilution with excess water and extraction of the cold reaction mixture with ethyl acetate, washing of the organic phase with bidistilled water, filtration through anhydrous Na_2SO_4 and dryness in vacuum, afforded a chromatographically pure sample (5 mg) of the detherification product gossypetin. ^1H NMR of **10**: δ ppm: 6.43 (*s*, H-6), 7.60 (broad singlet, H-2′), 6.89 (*d*, $J=8.0$ Hz, H-5′), 7.50 (*dd*, $J=8.0$ Hz & 2.5 Hz, H-6′), 3.71 (*s*, OMe), 3.73 (*s*, OMe). EI-MS of **10**: *m/z* (rel. int.): 346 (100%) $[M]^+$, 345 (95%) $[M–H]^+$, 328 (34%) $[M\text{-}18]^+$, 329 (32%) $[M\text{-}17]^+$, 182 (32%) $[A_1]^+$, 183 (24%) $[A_1+H]^+$, 137 (94%) $[B_2]^+$ and 109 (21%) $[B_2\text{-}28]^+$. ^{13}C NMR of **10**: Table 2.

3.2.3. Myricetin-3,5,3′,-trimethyl ether (*11*)

R_f-values (Table 1); UV λ_{max}^{MeOH} nm: Table 1; ESI-MS negative molecular ion: $m/z=359$ $[M–H]^-$ and an $[M\text{-}CH_3]^-$ ion at $m/z=344$; MS/MS: of the 344 ion: $[344+H\text{-}CH_3]^-$; at $m/z=330$; MS/MS of the 330 ion: $[330\text{-}CH_3]^-$ at $m/z=315$; complete acid hydrolysis (8 mg of **11** refluxed with 5 ml aqueous methanolic, 1:1, 2 N HCl, at 100 °C, for 7 h): no changes. Hydrolytic cleavage of **11**: 19 mg were refluxed with conc. HI (3 ml) and phenol (19 mg), over a heating mantel, for 0.5 h, followed by dilution with excess of saturated $NaHSO_3$ aqueous solution and extraction of the cold reaction mixture with ethyl acetate, washing of the organic phase with bidistilled water, filtration through anhydrous Na_2SO_4 and dryness in vacuum, afforded the detherification product, myricetin, **11a** (7 mg): R_f-values: Table 1; UV λ_{max}^{MeOH} nm: Table 1; ^1H NMR of **11**: δ ppm: 6.35 (*d*, $J=2.0$ Hz, H-6), 6.48 (*d*, $J=2.0$ Hz, H-8), 7.19 (broad s, H-2′), 7.20 (broad s, H-6′), 3.66 (*s*, OMe), 3.84 (*s*, OMe), 3.88 (*s*, OMe). EI-MS of **11**: *m/z* (rel. int.): 360 (100%) $[M]^+$, 359 (95%) $[M–H]^+$, 342 (30%) $[M\text{-}18]^+$, 343 (22%) $[M\text{-}17]^+$, 182 (27%) $[A_1]^+$, 183 (20%) $[A_1+H]^+$, 167 (94%) $[B_2]^+$ and 139 (21%) $[B_2\text{-}28]^+$. ^{13}C NMR of **11**: Table 2.

Acknowledgements

The authors wish to express their deep gratitude to Professor Dr. M. Linscheid, Institut für Chemie, Humboldt-Universität zu Berlin, Germany, for the ESI-MS measurements.

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