

Investigación

New Eremophilanoids from the Roots of *Psacalium radulifolium*. Hypoglycemic, Antihyperglycemic and Anti-Oxidant Evaluations

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Dedicated to Dr. Alfonso Romo de Vivar

Abstract. The investigation of the chemical constituents from the roots of *Psacalium radulifolium* (Compositae), a member of the matarique complex of medicinal plants, resulted in the isolation of four additional new eremophilanoids: radulifolin D, radulifolin E (ketodecompostin), radulifolin F (3 β -hydroxy-cacalone-3-*O*- β -D-glucopyranoside) and *epi*-radulifolin F (3 β -hydroxy-6-*epi*-cacalone-3-*O*- β -D-glucopyranoside), together with the known compounds maturinone, acetylmaturine, dimaturine, triacontanol, hydroxycacalolide, *epi*-hydroxycacalolide, β -sitosteryl-3-*O*- β -D-glucopyranoside, β -D-glucopyranose and saccharose. The methanol extract from the roots of this species displayed hypoglycemic activity, but cacalol, cacalone, *epi*-cacalone, *O*-methyl-1,2-dehydrocacalol and decompostin did not exhibit activity. The antihyperglycemic evaluation of the extract demonstrated that it was inactive. Some isolated compounds were also tested for antioxidant activity, and cacalol was found to be active.

Keywords: *Psacalium radulifolium*, Compositae, matarique, radulifolin D, radulifolin E, radulifolin F, *epi*-radulifolin F, eremophilanoids, hypoglycemic activity, antihyperglycemic activity, anti-oxidant activity.

Resumen. La investigación de los constituyentes químicos de las raíces de *Psacalium radulifolium* (Compositae), una especie perteneciente al complejo matarique de plantas medicinales, resultó en el aislamiento de cuatro nuevos eremofilanoides: radulifolina D, radulifolina E (cetodecompostina), radulifolina F (3-*O*- β -D-glucopiranosido de 3 β -hidroxycacalona) y *epi*-radulifolina F (3-*O*- β -D-glucopiranosido de 3 β -hidroxi-6-*epi*-cacalona), junto con las sustancias conocidas maturinona, acetil maturina, dimaturina, triacontanol, hidroxycacalólida, *epi*-hidroxycacalólida y 3-*O*- β -D-glucopiranosido de β -sitosterilo, β -D-glucopiranososa y sacarosa. El extracto metanólico de las raíces de esta especie mostró actividad hipoglucémica, pero cacalol, cacalona, *epi*-cacalona, el éter metílico de 1,2-deshidro-cacalol y la decompostina no mostraron actividad. La evaluación antihiper glucémica del extracto demostró su inactividad. La actividad anti-oxidante fue ensayada para algunas sustancias, y se encontró que el cacalol es activo.

Palabras clave: *Psacalium radulifolium*, Compositae, matarique, radulifolina D, radulifolina E, radulifolina F, *epi*-radulifolina F, eremofilanoides, actividad hipoglucémica, actividad antihiper glucémica, actividad anti-oxidante.

Matarique is the common name for a group of plants used in Mexican traditional medicine for the treatment of diabetes, kidney pains, infections, and general body pains, among other ailments [1-4]. This group includes *Psacalium decompositum*, *P. palmeri*, *P. peltatum*, *P. sinuatum*, and *A. thurberi*. *Psacalium* belongs to the Tussilaginoid genera of the Senecioneae (Compositae), and includes ca. 40 species which are located chiefly in Mexico [5]. The structures and chemistry of the secondary metabolites isolated from *P. decompositum* (syn: *Cacalia decomposita*) have been the subject of several investigations [6], and the synthesis of the main constituents, cacalol (**1**), cacalone (**2**) and structural analogs have been achieved [7]. Cacalol (**1**) has been found as the bioactive constituent in anti-microbial [8], antioxidant [9], allelopathic and phytopathogenic assays [10]. The hypoglycemic activity of extracts of *P. decompositum* and *P. peltatum* in mice have been evaluated [11,12], and the antihyperglycemic activity of aqueous extracts and some constituents from *P. decompositum* using diabetic mice have been determined [13].

P. radulifolium is considered a substitute for the preferred *P. decompositum* in the matarique complex of medicinal plants, and previous examination of the less polar constituents of the roots of this species allowed the isolation of **1**, **2**, *epi*-cacalone (**3**), radulifolin A (**4**), *epi*-radulifolin A (**5**), radulifolin B (**6**), radulifolin C (**7**), *O*-methyl-1,2-dehydrocacalol (**8**), adenostin A (**9**), decompostin (**10**) and neoadenostylone (**11**), from which **1** displayed major antimicrobial activity [14]. Here we report the hypoglycemic and antihyperglycemic evaluations of the methanol extract of the roots of *P. radulifolium*, and the isolation of the polar constituents, which resulted in the characterization of four new metabolites: radulifolin D (**12**), radulifolin E (ketodecompostin, **13**), radulifolin F (3 β -hydroxy-cacalone-3-*O*- β -D-glucopyranoside, **14**) and *epi*-radulifolin F (3 β -hydroxy-6-*epi*-cacalone-3-*O*- β -D-glucopyranoside, **15**), together with the known compounds maturinone (**16**), acetylmaturine (**17**), triacontanol, dimaturine (**18**), hydroxycacalolide (**19**), *epi*-hydroxycacalolide (**20**), β -sitosteryl- β -D-glucopyranoside, β -D-glucopyranose and sac-

charose. The anti-oxidant activity of some isolated compounds is also reported.

Results and discussion

Compound (**12**) was isolated as a yellow solid, and its HREIMS established the molecular formula $C_{15}H_{14}O_4$. UV spectrum showed bands of a conjugated ketone at $\lambda_{\max}$ 336, 277, 245, and 207 nm, and the IR spectrum revealed the presence of hydroxyl group (3583 cm^{-1}), conjugated carbonyl (1664 cm^{-1}) and multiple carbon-carbon bonds (1585 , 1463 cm^{-1}). The ^1H NMR spectrum (Table 1) showed considerable similarity with that of radulifolin C (**7**), a compound previously isolated from this species [14], establishing a close structural relationship. The most significant difference was the downfield shift and multiplicity of H-14 (δ 1.85), which appeared as a singlet, in comparison with the chemical shift of the same protons of radulifolin C (δ 1.43), which resonated as a doublet, indicating the presence of a hydroxyl at C-6 in **12**, in agreement with the molecular formula. ^{13}C NMR data showed the expected chemical shifts, and the assignments were corroborated by HMQC and HMBC experiments. Therefore this substance was 6-hydroxy-radulifolin C, and named radulifolin D (**12**). The 3-*O*-methyl derivative of **12** has been characterized from a chemical analysis of *Cacalia hastata* L. var. *tanakae* [15], but it was considered as an artifact due to the lack of optical activity. Radulifolin D (**12**) is dextrorotatory, while radulifolin C (**7**) is levorotatory. Considering that the twisting of the A/C rings is in the opposite direction to the pseudo-axial methyl group at C-6 (to

avoid interactions with the methyls at C-13 and C-15), the hydroxyl group at C-6 of radulifolin D could be tentatively proposed with the β - configuration (**12**), to explain the opposite specific rotation to that observed for radulifolin C (**7**).

Radulifolin E (**13**) was isolated as a UV active solid ($\lambda_{\max}$ 320, 280, 257 nm), with a molecular formula $C_{17}H_{18}O_5$ established from EIMS and NMR data. The IR spectrum contained bands at 1740 and 1668 cm^{-1} consistent with the presence of an acetate and an α,β -unsaturated ketone, respectively. The ^{13}C NMR spectrum (Table 2) showed 17 signals (four methyls, one methylene, four methines and eight quaternary carbons, including three carbonyls), and the chemical shifts and multiplicity observed in the ^1H NMR spectrum (Table 1) could be accounted by the furanoeremophilane skeleton with an acetate at C-6 similar to that of decompostin (**10**). The major difference between radulifolin E (**13**) and decompostin was the downfield chemical shift for H-1, due to the presence of a ketone at C-2. Therefore, radulifolin E (**13**) was established as 2-ketodecompostin, previously obtained as a derivative of decompostin via bromination (NBS) and oxidation (AgNO_3) [6d]. ^1H and ^{13}C NMR assignments (Tables 1 and 2) were confirmed by HMQC and HMBC experiments.

The FABMS, ^1H and ^{13}C NMR data for compounds **14** and **15** were consistent with the molecular formula $C_{21}H_{28}O_9$. An intense IR band at *ca.* 3400 cm^{-1} for both compounds suggested the presence of several hydroxyl groups, and bands at *ca.* 1660 cm^{-1} were ascribed to α,β -unsaturated carbonyl groups. ^1H and ^{13}C NMR spectra (Tables 1 and 2) indicated the presence of a β -D-glucopyranose fragment and a cacalone aglycon. The anomeric hydrogen of **14** was observed at δ 4.42 and the analysis of the COSY spectrum determined the sequen-

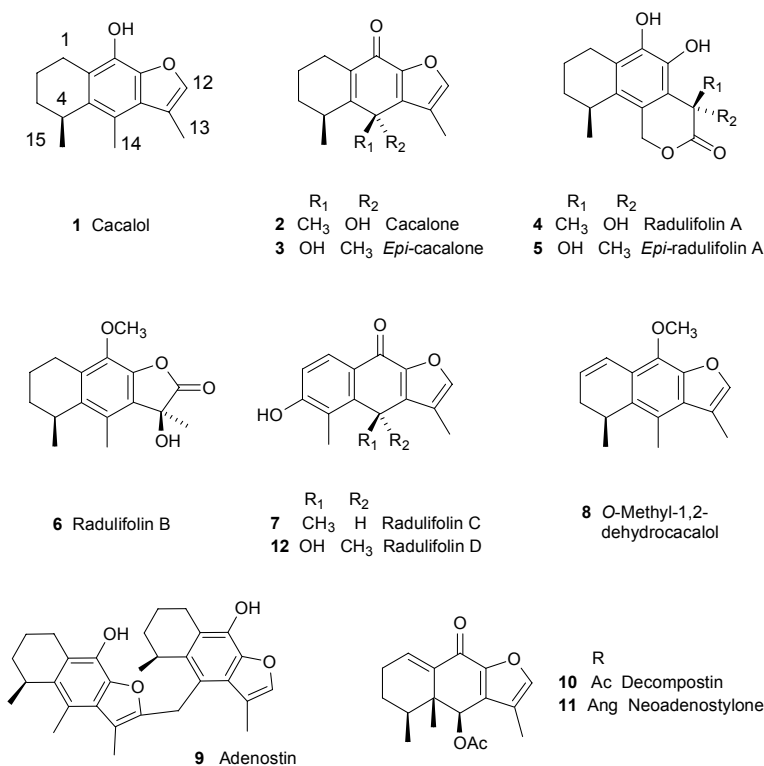


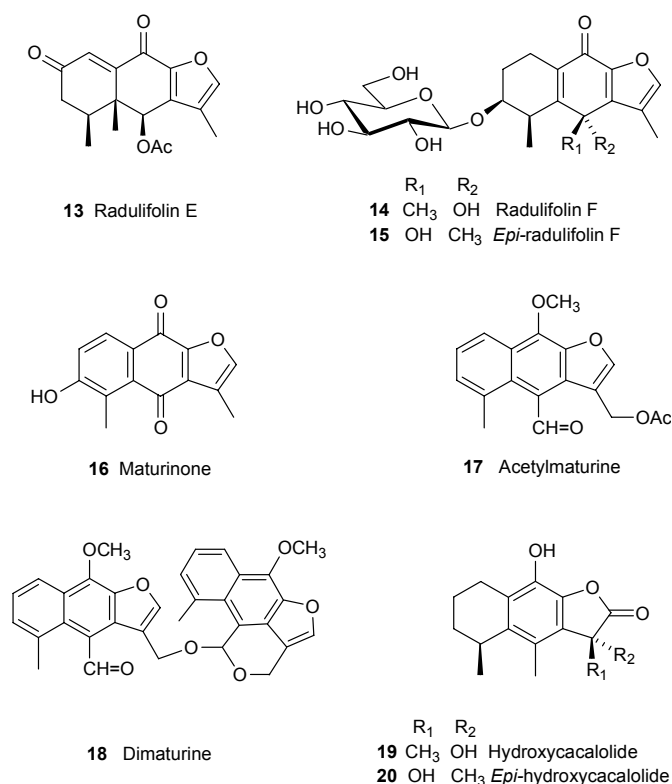
Table 1. ¹H NMR (500 MHz) Chemical Shift Assignments for Compounds **12-15**.

H	12 ^a	13 ^b	14 ^c	15 ^c
1	8.05 d (8)	6.83 s	2.39 m	2.38 dd (3)
2a	6.95 d (8)		1.99 m	2.0 m
2b			1.87 m	1.79 m
3a		2.41 d (11)	4.03 ddd (3,3,3)	4.03 ddd (3,3,3)
3b		2.38 dd (11, 5)		
4		2.54 qd (7, 5)	3.37 dq (7, 3)	3.02 qd (7,3)
6		6.43 s		
12	7.43 q (1.5)	7.53 q (1)	7.52 q (1)	7.50 q (1)
13	2.31 d (1.5)	1.97 d (1)	2.21 d (1)	2.21 (1)
14 α	1.85 s	1.31 s	1.63 s	1.60 s
14 β				
15	2.65 s	1.08 d (7)	1.21 d (7)	1.22 d (7)
OCOCH ₃		2.25 s		
1'			4.42 d (8)	4.44 d (8)
2'			3.01 dd (8, 8)	3.07 dd (9,8)
3'			3.31 dd (9,8)	3.37 dd (9,9)
4'			3.17 dd (9,9)	3.29 dd (9,9)
5'			3.32 m	3.31 m
6'a			3.52 m	3.65 m
6'b			3.78 m	3.84 m
-OH			4.13 br s	4.18 br s
			4.39 br s	4.26 br s
				4.51 br s

^a Recorded in CDCl₃ + DMSO. ^b Recorded in CDCl₃. ^c Recorded in CD₃COCD₃. J values (in Hz).**Table 2.** ¹³C NMR Chemical Shifts Assignments for Compounds **12-15**.

Position	12 ^a	13 ^b	14 ^c	15 ^c
C-1	126.45 (d)	129.715 (d)	18.36 (t)	18.44 (t)
C-2	114.30 (d)	198.11 (s)	20.88 (t)	20.40 (t)
C-3	161.03 (s)	43.26 (t)	79.15 (d)	77.34 (d)
C-4	124.28 (s)	38.43 (d)	33.88 (d)	36.00 (d)
C-5	148.14 (s)	47.53 (s)	158.81 (s)	161.18 (s)
C-6	71.216 (s)	72.63 (d)	70.70 (s)	72.43 (s)
C-7	142.82 (s)	137.065 (s)	142.34 (s)	142.53 (s)
C-8	144.345 (s)	146.415 (s)	146.09 (s)	146.07 (s)
C-9	172.99 (s)	173.45 (s)	174.88 (s)	175.05 (s)
C-10	123.65 (s)	156.35 (d)	131.02 (s)	129.94 (s)
C-11	120.90 (s)	121.59 (d)	121.52 (s)	121.40 (s)
C-12	145.08 (d)	147.76 (s)	145.14 (d)	144.83 (d)
C-13	8.956 (q)	8.446 (q)	9.01 (q)	8.86 (q)
C-14	27.46 (q)	14.38 (q)	27.39 (q)	28.15 (q)
C-15	13.44 (q)	16.904 (q)	20.32 (q)	20.89 (q)
OCOCH ₃		21.271 (q)		
OCOCH ₃		170.374 (s)		
C-1'			102.71 (d)	102.24 (d)
C-2'			74.78 (d)	74.91 (d)
C-3'			77.65 (d)	77.87 (d)
C-4'			71.64 (d)	71.83 (d)
C-5'			77.65 (d)	77.40 (d)
C-6'			62.97 (t)	63.08 (t)

^a Recorded at 125 MHz in CDCl₃ + DMSO. ^b Recorded at 75 MHz in CDCl₃.^c Recorded at 125 MHz in CD₃COCD₃ at 25 °C. Multiplicity (in parenthesis) deduced by DEPT.



tial vicinity for the carbinolic hydrogens of the β -D-glucopyranose (δ 3.78 – δ 3.01), which correlated with the corresponding signals at δ 74.78 (C-2'), 77.65 (C-3'), 71.64 (C-4'), 77.60 (C-5'), 62.97 (C-6') in the HMQC spectrum. The crosspeaks in the HMBC spectrum between H-3 (δ 4.03) and H-2' (δ 3.01) with C-1' (δ 102.71) confirmed that the glucopyranose was bound to C-3 of the modified eremophilane. NOESY interactions between H-1' and H-3, and between H-3 and H-4, confirmed the stereochemical assignments. Similar crosspeaks were observed for **15**, and therefore, the difference between the two substances was in the aglycon fragment. The stereochemistry at C-6 was deduced by comparing the ^1H NMR data of **14** and **15** with those of **2** and **3** [7d]. Specifically, the downfield shift of H-4 in **14** (δ 3.37) with respect to that of H-4 in **15** (δ 3.18) was in agreement with the α - and β - orientation of the hydroxyl groups, respectively [16].

The methanol extract obtained directly from the roots of *P. radulifolium* was evaluated as hypoglycemic agent in normoglycemic rats, following the standard procedures [17], and the results are shown in Table 3. This residue showed significant decrease of blood glucose concentration ($p < 0.05$) at 7 h using several doses (30, 100 and 300 mg/kg), without returning to the basal blood glucose level. The effect of the hypoglycemic model drug is also included (glybenclamide, 10 mg/kg). Cacalol (**1**), The mixture of **2** + **3**, *O*-methyl-1,2-dehydrocacalol (**8**), and decompostin (**10**) did not display significant hypoglycemic activity at doses of 3.1, 10 and 31 mg/kg.

The antihyperglycemic effect of the methanol extract was also tested at the same doses using male diabetic Wistar rats,

diabetized via streptozotocin injection, following the standard procedures. The results obtained indicated that this residue did not display significant antihyperglycemic effect in comparison with the model drug.

It has been proposed that the anti-oxidant activity may play a role in the antihyperglycemic and hypoglycemic activities [18], and some reactive oxygen species (superoxide anion, hydrogen peroxide and hydroxyl free radical) are involved in the physiology of several diseases, including diabetes [19]. Therefore, the antioxidant activity of some isolated compounds (cacalol (**1**), the mixture of cacalone and *epi* cacalone (**2** + **3**), radulifolin C (**7**), neoadenostylone (**11**), radulifolin D (**12**) and radulifolin F (**14**)) was evaluated *via* the interaction with the stable free radical DPPH, following the procedures described in the experimental section. The results are included in Table 4, and they showed that only cacalol (**1**) exhibited significant activity, in agreement with previous reports [20].

It is interesting to note that some pure secondary metabolites did not display hypoglycemic effect, while the activity the methanolic extract of the roots of *P. radulifolium* was evident. Although the mechanism of action of the eremophilanes, modified eremophilanes or other natural products [21] in changing the blood glucose levels remains unknown, the described results suggest that there is some correlation with the ethnomedical use of the plant as antidiabetic agent. However, this plant, as all the plants used in traditional medicine, should not be used until safety studies are completed.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Plus-500 instrument, and the chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane. Infrared spectra were recorded with a Nicolet Magna IR TM 750 and Perkin Elmer 283B instruments. MS data were recorded with a JEOL JMS-AX 505 HA mass spectrometer. Electron impact mass spectra were obtained at 70 eV ionization energy. Vacuum chromatography was performed on Merck Kieselgel 60 (0.040-0.863 mm). TLC analyses were performed on TLC plates with Si gel 60 F₂₅₄ (Merck) or ALUGRAM® SIL G/UV₂₅₄ silica gel plates. The compounds were detected by their absorbance under UV₂₅₄ and UV₃₆₆, or with a charring solution (12 g of ceric ammonium sulfate dihydrate, 22.2 mL of concentrated H₂SO₄ and 350 g of ice). Solvents were distilled prior to use.

Plant material. The roots of *P. radulifolium* (HBK) H. Rob. & Brettell were collected in San Luis Potosí in 1995. Plant material was identified by Dr. Robert Bye and M. Sc. Edelmira Linares from the Instituto de Biología de la UNAM. The voucher Bye & Linares 20028 was deposited in the Ethnobotanical Collection, and the voucher Bye & Linares 20149 was deposited in the National Herbarium, of the Instituto de Biología de la UNAM.

Table 3. Hypoglycemic Effect (Variation of Percentage Values) of the Methanol Extract of the Roots of *P. radulifolium* (Oral administration).

Time (h)	Glibenclamide ^a	Doses (extract)		
	10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
1.5	-15.68 ± 3.66*	-0.56 ± 5.87	-3.11 ± 3.91	-0.39 ± 4.66
3	-31.56 ± 7.75*	-3.95 ± 6.52	0.16 ± 7.19	0.03 ± 4.75
5	-35.79 ± 6.74*	-12.74 ± 2.97	-10.10 ± 5.70	-11.80 ± 3.43
7	-35.75 ± 4.33*	-21.70 ± 1.77*	-12.92 ± 8.51	-21.66 ± 3.09*

^a Glibenclamide was used as positive control. *Significative values ($p < 0.05$).

The values represent the mean ± standard deviations from five independent experiments.

The negative value (-) indicates a decrease in glucemia.

Extraction and isolation. Previously we reported preparation of the *n*-hexane, CH₂Cl₂-EtOH (3:2) and MeOH extracts from the roots of this species, and the chemical analysis of the less polar residue [14]. The CH₂Cl₂-EtOH (3:2) extract (10 g) was loaded onto a column chromatography which was developed under reduced pressure using a gradient of *n*-hexane-EtOAc as elution system, to afford seven main fractions (named H to N). Fractions H and I were combined (31.2 mg), and this mixture was applied to a preparative TLC which was eluted with *n*-hexane-EtOAc (20:1), to yield maturinone [6b] (**16**, 5.6 mg), acetylmaturine [22] (**17**, 1.7 mg), and triacontanol (9 mg). Column rechromatography over silica gel of fraction J (350 mg) using *n*-hexane-EtOAc gradient as elution system afforded dimaturine [23] (**18**, 2.6 mg). Fractions K (15 mg) and L (1.3 g) were combined and subjected to Si-gel column chromatography using *n*-hexane-EtOAc (4:1) as elution system, affording decompostine [6d] (**10**, 896.5 mg). Fraction M (3.135 g) was chromatographed over Si gel using *n*-hexane-EtOAc gradient, to give several fractions. Some of these fractions were purified by column chromatography on Si gel using *n*-hexane-EtOAc gradient, leading to a mixture hydroxycacalolide [13a] (**19**) and *epi*-hydroxycacalolide [13a] (**20**, 3.3 mg), cacalone (**2**) and 6-*epi*-cacalone (**3**, 20 mg), radulifolin C [14] (**7**, 2.7 mg), radulifolin D (**12**, 7.1 mg), radulifolin E (ketodecompostine, **13**, 2.2 mg). Column chromatography over Si gel of fraction N (2.56 g) using *n*-hexane-EtOAc as gradient elution system yielded β-sitosterol 3-*O*-β-D-glucopyranoside, **14**, 8.9 mg, radulifolin F (3-β-hydroxycacalone-3-β-*O*-D-glucopyranoside, **14**, 13.2 mg) and *epi*-radulifolin F (3-β-hydroxy-6-*epi*-cacalone-3-β-*O*-D-glucopyranoside, **15**, 26.3 mg) and β-D-glucopyranose. From the polar fractions of the methanolic extract (fractions Ñ to S) were identified *O*-methyl-1,2-dehydrocacalol [6e] (**8**), cacalol (**1**), decompostin (**10**), β-sitosterol, stigmaterol, cacalone (**2**) and 6-*epi*-cacalone (**3**), saccharose and β-D-glucopyranose.

Radulifolin D (12). 7.1 mg, yellow solid mp 122-124 °C, Rf: 0.226 (7:3 hex-AcOEt), mp 122-124 °C, $[\alpha]_D^{20} = +30.0$ (c 0.05, MeOH); UV $\lambda_{\max}$ (log ϵ): 207 (4.50), 245 (4.20), 277 (4.00), 336 (4.00); IR (CHCl₃, cm⁻¹): 3583, 3268, 2928, 2854, 1762, 1664, 1585, 1463, 1419, 1354, 1288, 1156, 1113, 996, 996, 918; ¹H and ¹³C NMR data, see Tables 1 and 2; EIMS:

C₁₅H₁₄O₄, 258 [M⁺] (26), 243 (100), 240 (7), 215 (10), 201 (3), 187 (3), 85 (3), 157 (3), 135 (6), 128 (6), 115 (8), 109 (11), 91 (3), 77 (6), 55 (3), 43 (6).

Radulifolin E (ketodecompostin, 13). 4.4 mg, yellow solid mp 222-225 °C (lit. [6d] 220-221 °C), Rf: 0.413 (3:2 hex-AcOEt); Mp. 222-225 °C; UV $\lambda_{\max}$ (log ϵ): 319.5 (3.95); 280.5 (3.58); 257 (3.71); 240.5 (3.65); 232 (3.66); 224.5 (3.65); 205.5 (3.72); IR (CHCl₃, cm⁻¹): 3037, 2971, 2940, 1747, 1672, 1606, 1531, 1463, 1415, 1372, 1315, 1176, 1050, 1030, 983, 928; ¹H and ¹³C NMR data, see Tables 1 and 2; EIMS: C₁₇H₁₈O₅, 302 [M⁺] (10), 274 (0.5), 260 (64), 242 (100), 227 (15), 214 (9), 199 (14), 191 (24), 163 (5), 161 (5), 137 (20), 123 (810), 115 (9), 109 (8), 91 (7), 77 (7), 65 (4), 53 (8), 43 (32), 41 (5).

Radulifolin F (3β-hydroxycacalone-3-*O*-β-D-glucopyranoside, 14). 13.2 mg, yellow oil; Rf: 0.295 (85:15 hex-AcOEt); IR (CHCl₃, cm⁻¹): 3401, 2936, 1660, 1619, 1603, 1535, 1459, 1445, 1421, 1363, 1162, 1079, 1035, 923, 887; ¹H and ¹³C NMR data, see Tables 1 and 2; FABMS⁺: C₂₁H₂₈O₉, 447 [M⁺ + Na] (58), 407 (20), 263 (48), 245 (100), 227 (72), 191 (24), 154 (31), 136 (28), 91 (20), 77 (19), 44 (17).

Epi-radulifolin F (3β-hydroxy-6-*epi*-cacalone-3-*O*-β-D-glucopyranoside, 15). 26.3 mg yellow oil; Rf: 0.295 (85:15 hex-AcOEt); IR (KBr, cm⁻¹): 3411, 2930, 1654, 1614, 1535, 1451, 1422, 1369, 1256, 1224, 1201, 1164, 1078, 1038, 936, 888, 814, 623, 595, 532; ¹H and ¹³C NMR data, see Tables 1 and 2;

Table 4. Percentage of Inhibition of the Free Radical (DPPH) by Compounds **1-3**, **7**, **11**, **12** and **14**.

Compound	Concentrations		
	1 μM	10 μM	100 μM
1	15.27	47.70	73.13
2 + 3	8.91	13.60	26.98
7	9.47	11.72	14.01
11	6.77	10.23	25.79
12	8.8	9.09	16.76
14	5.06	11.37	19.55

FABMS⁺: C₂₁H₂₈O₉, 447 [M⁺ + Na] (100), 425 (17), 399 (8), 371 (38), 263 (49), 245 (44), 227 (26), 191 (21), 177 (35), 154 (59), 136 (51), 91 (26), 77 (25), 55 (24), 41 (25), 23 (58).

Biological evaluations. The methanol extract used for biological assays was obtained by direct maceration of the dried roots of the plant at room temperature (1 L per each 100 g) by 48 h two times. Male Wistar normoglycaemic rats of 60-65 days old, generally weighing 200-250 g, were used. The animals were housed under standard laboratory conditions and maintained on standard pellet diet and water ad libitum. Rats were placed in single cages with wire-net floors and deprived of food for 18 h before experimentation but allowed free access to tap water throughout. All experiments were carried out using 5 animals per group. Male Wistar rats were made diabetic by an intraperitoneal injection of streptozotocin (60 mg/kg) in citrate buffer, pH 6.3 [24]. Extracts were suspended in 0.05 % of Tween 80 in saline solution. Glibenclamide (10 mg/kg) was used as a hypoglycemic model drug [25]. All extracts were prepared freshly immediately before the experimentation and administered by intragastrical route at 30, 100 and 300 mg/kg. Control rats received the vehicle (0.05 % Tween 80) in the same volume (0.5 mL/100 g) by the same route. Blood samples were collected from caudal vein by means of a little incision in the end of the tail at 0, 1.5, 3, 5, 7 and 9 h after drug administration. Blood glucose concentration was estimated by enzymatic glucose oxidase method using a commercial glucometer (One Touch Basic I, Jonhsons-Johnsons). The percentage variation of glycemia for each group was calculated with respect to initial (0 h) level according to:

$$\% \text{ variation of glycemia} = [(G_t - G_i) / G_i] \times 100,$$

Where G_i was initial glycemia values and G_t was the hypoglycemia value at +1.5, +3, +5, and +7 h, respectively [17b]. Statistical significance was estimated by analysis of variance (ANOVA) followed by Dunnett's test t . $p < 0.05$ implies significance.

Evaluation of antioxidant activity. The potential antioxidant activity of plant extracts and pure compounds was assessed on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical [26]. Reaction mixtures containing test samples (dissolved in ethanol, at 1, 10 and 100 μ M) and DPPH ethanolic solution (66.66 μ M) in amber vials (4 mL) were stirred for 30 min, and absorbances were measured at 515 nm. Percent of inhibition by sample treatment was determined by comparison with a control group [27].

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