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Phenylethanoids, iridoids and a spirostanol saponin from *Veronica turrilliana*

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Abstract

From the aerial parts of *Veronica turrilliana* two phenylethanoid glycosides, turrilliosides A and B and a steroidal saponin, turrillianoside were isolated and their structures elucidated as β-(3,4-dihydroxyphenyl)ethyl-4-*O*-*E*-caffeoyl-*O*-[β-glucopyranosyl-(1 → 4)-α-rhamnopyranosyl-(1 → 6)]-β-glucopyranoside, β-(3,4-dihydroxyphenyl)ethyl-4-*O*-*E*-caffeoyl-[6-*O*-*E*-feruloyl-β-glucopyranosyl-(1 → 4)-α-rhamnopyranosyl-(1 → 6)]-β-glucopyranoside and (23*S*,25*S*)-12β,23-dihydroxyspirost-5-en-3β-yl *O*-α-rhamnopyranosyl-(1 → 4)-β-glucopyranoside, respectively. Furthermore, eight known glucosides are reported namely, catalpol, catalposide, verproside, amphicoside, isovanilloylcatalpol, aucubin, arbutin, and 6-*O*-*E*-caffeoylarbutin, the latter two for the first time in the genus *Veronica*. The two phenylethanoid glycosides were found to be potent DPPH radical scavengers. All of the tested compounds were inactive against the representative species of fungi and bacteria.

Keywords: *Veronica turrilliana*; Plantaginaceae; Phenylethanoid glycosides; Spirostanol saponin; Iridoid glucosides; Arbutin; Radical scavenging

1. Introduction

Veronica turrilliana Stoj. & Stef. is a perennial herb endemic to Strandzha, a mountain on the territory of Bulgaria and Turkey with strong links to the Caucasian flora. The preliminary investigations revealed that this species had a chemical profile different from the other representatives of subgenus *Pentasepalae* (*V. teucrium*, *V. austriaca*, and *V. rhodopaea*). The TLC analysis of extracts of *V. turrilliana* showed presence of five iridoid glycosides common to *Veronica* (Taskova et al., 2004) and traces of the flavonoids apigenin and apigenin 4′-methyl ether (Nikolova et al., 2005). The main secondary metabolites of the species, however, remained unidentified.

As a continuation of our work on the chemistry of genus *Veronica* (Plantaginaceae) (Taskova et al., 2002, 2004, 2006), we performed a phytochemical study of the endemic species *V. turrilliana.* Isolation and characterization of two new phenylethanoids and a steroidal saponin together with eight known compounds are reported. The major constituents in the methanol extract, arbutin (**10**) and its derivative **11**, along with the two novel phenylethanoids (**1**–**2**) were evaluated for their radical scavenging potential. Antifungal and antibacterial activities of **1**–**3**, **10** and **11** were also tested.

2. Results and discussion

The water-soluble part of the methanolic extract of *Veronica turrilliana* afforded two novel phenylethanoid glycosides **1**–**2** and a steroidal saponin **3**, as well as the known compounds **4**–**11** (Fig. 1).

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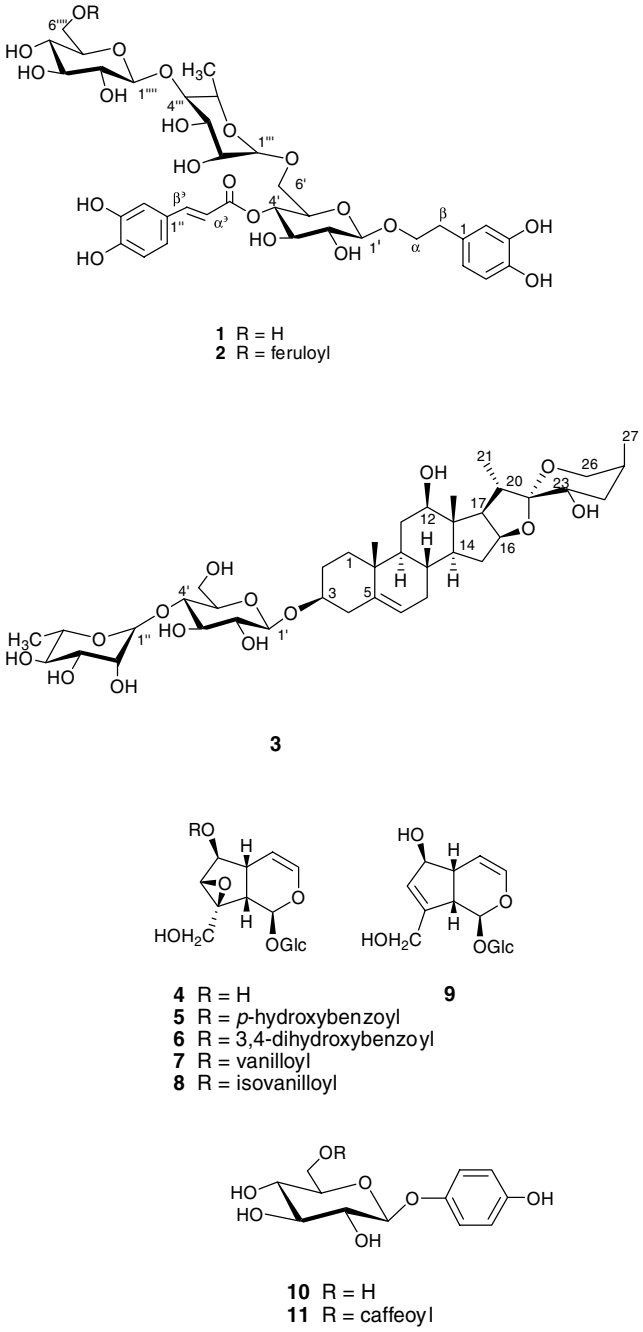

Fig. 1. Compounds **1-11** isolated from *Veronica turrilliana*

Table 1
NMR data for compounds **1** and **2** in CD_3OD[a]

Position	**1**		**2**	
	H[b]	C[c]	H[b]	C[c]
Aglycone				
α	3.97 *m*, 3.75[d] *m*	72.5 *t*	3.95 *m*, 3.75[d] *m*	72.4 *t*
β	2.80 *t* (7.4)	36.7 *t*	2.79 *t* (7.0)	36.7 *t*
1		131.4 *s*		131.4 *s*
2	6.69 *d* (1.9)	117.2 *d*	6.69 *d* (2.1)	117.2 *d*
3		146.1 *s*		146.1 *s*
4		144.7 *s*		144.7 *s*
5	6.68 *d* (8.1)	116.6 *d*	6.68 *d* (8.1)	116.5 *d*
6	6.56 *dd* (8.1/1.9)	121.4 *d*	6.56 *dd* (8.1/2.1)	121.3 *d*
Glucosyl				
1′	4.40 *d* (7.7)	104.2 *d*	4.30 *d* (7.8)	104.2 *d*
2′	3.68[d]	74.5 *d*	3.61[d]	74.6 *d*
3′	3.50[d]	75.1 *d*	3.48[d]	75.4 *d*
4′	5.0	70.7 *d*	4.95	70.3 *d*
5′	3.4 *m*	74.0 *d*	3.30[d]	74.0 *d*
6′	3.75[d], 3.50[d]	67.6 *t*	3.75[d], 3.48[d]	67.5 *t*
Rhamnosyl				
1‴	4.63 *d* (1.4)	102.3 *d*	4.59 *d* (1.6)	102.3 *d*
2‴	3.84 *dd* (3.4/1.6)	72.0 *d*	3.82[d]	72.0 *d*
3‴	3.68[d]	72.3 *d*	3.65[d]	72.3 *d*
4‴	3.96 *t* (8.3)	84.0 *d*	3.88[d]	83.7 *d*
5‴	3.58 *m*	69.9 *d*	3.59	69.9 *d*
6‴	1.21 *d* (6.2)	18.1 *q*	1.18 *d* (6.2)	18.0 *q*
Glucosyl				
1⁗	4.53 *d* (7.6)	105.8 *d*	4.58 *d* (7.8)	105.5 *d*
2⁗	3.14[d] *t* (9.1)	76.0 *d*	3.18[d]	75.8 *d*
3⁗	3.31[d]	77.7 *d*	3.37[d]	77.8 *d*
4⁗	3.21[d]	71.2 *d*	3.30[d]	71.5 *d*
5⁗	3.20[d]	78.0 *d*	3.35–3.55[d]	75.2 *d*
6⁗	3.69[d], 3.52[d]	62.4 *t*	4.28	64.4 *t*
Caffeoyl				
CO		168.4 *s*		168.3 *s*
α′	6.32 *d* (15.9)	115.2 *d*	6.30 *d* (15.9)	115.4 *d*
β′	7.59 *d* (15.9)	147.6 *d*	7.53 *d* (15.9)	147.5 *d*
1″		127.7 *s*		127.7 *s*
2″	7.08 *d* (1.9)	115.3 *d*	7.01 *d* (1.8)	115.1 *d*
3″		146.9 *s*		146.8 *s*
4″		149.8 *s*		149.6 *s*
5″	6.79 *d* (8.3)	116.4 *d*	6.81 *d* (8.2)	116.4 *d*
6″	6.98 *dd* (8.3/1.9)	123.3 *d*	6.89 (8.2/1.8)	123.2 *d*
Feruloyl				
CO				169.1 *s*
α″			6.25 *d* (15.9)	115.5 *d*
β″			7.52 *d* (15.9)	146.9 *d*
1⁗				127.4 *s*
2⁗			7.12 *d* (1.8)	111.8 *d*
3⁗				149.4 *s*
4⁗				150.6 *s*
5⁗			6.68 *d* (8.0)	116.4 *d*
6⁗			7.04 *dd* (8.0/1.8)	124.2 *s*
OMe			3.88 *s*	56.5 *q*

[a] Assignments were established by DEPT, HSQC and HMBC data.
[b] 250 MHz, chemical shifts in ppm relative to TMS; *J* in Hz.
[c] 62.5 MHz; multiplicity was established from DEPT data.
[d] Overlapped signals.

Positive ferric chloride tests of **1** and **2** indicated their phenolic nature. Initial inspection of **1** and **2** by ^1H NMR spectra revealed the common key features suggestive of phenylethanoids: signals (δ 6.5–6.7) for an aromatic ABX spin system and the presence of two mutually coupled methylene groups (δ 2.8–4.0) arising from a β-(3,4-dihydroxyphenyl)ethyl moiety, and three anomeric protons assignable to two glucosyl and one rhamnosyl moieties (Table 1).

Compound **1**, turrillioside A, was isolated as an amorphous powder. The molecular formula of **1** was determined as $C_{35}H_{46}O_{20}$ by high resolution (HR)-FAB mass spectrometry. DEPT experiments indicated the presence of one methyl, four methylene, 23 methine and seven quaternary carbons. As stated above the signals for ABX aromatic protons (δ 6.56, 6.68, 6.69), oxygenated methylene

protons (δ 3.75, 3.97) and methylene protons (δ 2.80) were assigned to the 3,4-dihydroxyphenylethanol moiety. Another set of signals of an aromatic ABX system (δ 6.79, 6.98, and 7.08) and a *trans*-α,β-unsaturated olefin (δ 6.32, 7.59, each *d*, $J = 15.9$ Hz) suggested the presence of a (*E*)-caffeoyl moiety. The NMR spectra indicated the presence of three sugar moieties [δ_H 4.40 (1H, *d*, $J = 7.7$ Hz), 4.53 (1H, *d*, $J = 7.6$ Hz), 4.63 (1H, *d*, $J = 1.4$ Hz); δ_C 104.2, 105.8 and 102.3] which were identified as two glucopyranosyl and one rhamnopyranosyl units. Acid hydrolysis of **1** gave glucose and rhamnose, identified by TLC with authentic samples.

The chemical shifts of **1** were similar to that of forsythoside A (Endo et al., 1981), except that **1** showed additional signals for a second glucosyl moiety and a downfield shift of C-4‴ of the rhamnose unit (δ 84.0). The HMBC experiments showed correlations from H-1⁗ (δ 4.53, *d*, $J = 7.6$ Hz) to C-4‴ and from H-1‴ (δ 4.63, *d*, $J = 1.4$ Hz) to the deshielded C-6′ (δ 67.6), which established the sequence of the disaccharide unit to be Glc-(1 → 4)-Rhm and its attachment to C-6′ of the inner glucose. Consequently, compound **1** appeared to be a positional isomer of rossicaside A (Lin and Chen, 2004) and its structure was elucidated to be β-(3,4-dihydroxyphenyl)ethyl-4-*O*-*E*-caffeoyl-*O*-[β-glucopyranosyl-(1 → 4)-α-rhamnopyranosyl-(1 → 6)]-β-glucopyranoside. The configurations of the anomeric carbons of the sugars were deduced from the coupling constants and chemical shift values by comparison with published data (Agrawal et al., 1985).

Compound **2**, turrillioside B, was isolated as an amorphous powder. The molecular formula of **2** was determined as $C_{45}H_{54}O_{23}$ by HR-FABMS. DEPT experiments indicated the presence of two methyl, four methylene, 28 methine and 11 quaternary carbons. A close structural similarity of **1** and **2** was evident from a comparison of the ^{1}H and ^{13}C NMR data, except the presence of an additional (*E*)-feruloyl moiety in **2** (Table 1). The NMR spectra of **1** and **2** were almost identical, except for the downfield shift of H_2-6 of the terminal glucose (δ 4.28), and the ^{13}C NMR signals attributable to C-5 and C-6 of the terminal glucose which were shifted by −2.8 and 2.0 ppm, respectively, consistent with acylation by a feruloyl moiety of OH at C-6. The HMBC correlations confirmed the attachment of the feruloyl moiety to position C-6⁗ of the terminal glucose: C=O (δ 169.1) to H_2-6⁗ (δ 4.28), H-β″ (δ 7.52) and H-α″ (δ 6.25); O$\underline{C}H_3$ (δ 3.88) to C-3⁗′ (δ 149.4), C-3⁗′ to H-2⁗′ (δ 7.12) and H-2⁗′ to C-β″ (δ 146.9) and C-6⁗′ (δ 124.2). The characteristic ion at *m*/*z* 623 [M-177-162] formed after fragmentation of *m*/*z* 962 in negative ESI/MS/MS produced after sequential expulsion of feruloyl and glucosyl and the lack of ion at *m*/*z* 637 [M-163-162] (sequential expulsion of caffeoyl and glucosyl) were in agreement with the position of the feruloyl moiety at the terminal glucose unit. Consequently, the structure of compound **2** was elucidated to be β-(3,4-dihydroxyphenyl)ethyl-4-*O*-*E*-caffeoyl-[6-*O*-*E*-feruloyl-β-glucopyranosyl-(1 → 4)-α-rhamnopyranosyl-(1 → 6)]-β-glucopyranoside.

Compound **3**, named turrillianoside was identified as a spirostanol saponin with molecular formula of $C_{39}H_{62}O_{14}$ as determined by the positive-ion HR-FABMS. The ^{1}H and ^{13}C NMR signal assignments were made from DEPT and 2D NMR spectra including COSY, HSQC, ROESY, TOCSY and HMBC spectra (Table 2). DEPT experiments indicated the presence of five methyl, nine methylene, 21 methine and four quaternary carbons. The ^{1}H NMR spectrum of **3** displayed characteristic signals for steroid methyl protons at δ 0.85 (*s*, H_3-19), 1.23 (*s*, H_3-18), 1.48 (*d*, $J = 6.9$ Hz, H_3-21) and 1.10 (*d*, $J = 7.2$ Hz, H_3-27), an olefinic proton at δ 5.28 (*d*, $J = 5.0$ Hz, H-6) and two anomeric protons at δ 4.93 (1H, *d*, $J = 7.8$ Hz) of glucopyranosyl and 5.92 (1H, *bs*) of rhamnopyranosyl units. Acid hydrolysis of **3** gave glucose and rhamnose (TLC). The above data along with the characteristic acetal carbon signal at δ 112.5 and two anomeric carbon signals observed at δ 102.5 and 102.2 led to the suggestion that **3** was a spirostanol diglycoside.

The ^{1}H NMR spectrum of the aglycone part showed six protons geminal to oxygenated function: doublets at δ 3.57 and 3.31, double doublets at δ 4.66 and 4.04 and two multiplets at δ 3.83 and 4.10. Five carbinol signals at δ 81.6, 78.9, 77.98, 64.0 and 63.1 apart from the acetal carbon at δ 112.5 were observed in the ^{13}C NMR. These data are in accordance with a 3β-hydroxyspirostane skeleton of the aglycone with two additional oxymethine carbons at δ 78.9 and 63.1. The ROESY and TOCSY experiment showed correlations between the doublet methyl Me-21 at δ 1.48 and the methine H-20 at δ 3.21, the latter with the proton H-17 at δ 2.25, which showed a cross peak with the proton H-16 at δ 4.66. The correlations in the HMBC experiment of C-5/H-19, C-12/H-11, H-17 and H-18, C-22/H-20, H-21 and H-26, C-23/H-20 and H-24, suggested the aglycone of **3** to be spirost-5-ene-3,12,23-triol. To our knowledge a compound with this pattern of hydroxylation of the spirost-5-ene skeleton has not been reported; however, a comparison with the literature data for compounds with identical partial structures supported these assignments. Thus, the spectral data for the DEF part of paniculogenin, (23*S*, 25*S*)-spirostane-3,23-diol (Agrawal et al., 1985), coincide with those of **3**, whilst that of bahamgenin, spirost-5-ene-3,12,15-triol (Coll et al., 1983), matched well with the rings ABC. The HMBC correlations between the proton at δ 1.93 (H-24) with both carbons at δ 63.1 (C-23) and δ 30.3 (C-25), the protons at δ 1.10 (H_3-27) with the carbons at δ 30.3, 35.8 and 64.0 (C-25, C-24 and C-26), and the proton at δ 4.04 (H-26) with the acetal carbon at δ 112.5 (C-22) and the methyl at δ 17.4 (C-27) were in accordance with a hydroxyl group at C-23.

The stereochemistry of the C-12 and C-23 carbons was defined by COSY and ROESY experiments. The H-12 proton at δ 3.57 showed a spatial proximity with the 9α, 11α, 14α and 17α protons justifying β-orientation for the hydroxyl group on the C-12 carbon. The shielding effect of this group on C-18 (δ 10.9), compared to that of an α-OH ($\delta_{C\text{-}18}$ ca. 18 ppm, Osorio et al., 2005) is in line with the

Table 2
NMR[a] spectral data for compound **3** in pyridine-d_5 and HMBC correlations

Position	H[b]	C[c]	HMBC (C → H)
1	ax 0.92 *dt* (2.5, 13.0, 12.0)	37.2 *t*	H-19
	eq 1.66 *m*		
2	ax 1.64 *m* eq 1.84 *m*	31.2 *t*	
3	3.83 *m*	77.98 *d*	H-1′
4	ax 2.38 *m*	39.0 *t*	
	eq 2.68 *ddd* (1.2, 5.8, 13.5)		
5		140.6 *s*	H-19
6	5.28 *d* (5.0)	121.6 *d*	
7	ax 1.85 *m*	31.6 *t*	
	eq 2.06 *m*		
8	1.52 *d* (4.4)	30.5 d	H-6
9	1.03 *dt* (4.6, 12.2, 12.0)	49.8 *d*	H-11, H-19
10		36.1 *s*	H-6
11	1.63 *m*	29.9 *t*	
	1.99 *m*		
12	3.57 *d* (0.85)	78.9 *d*	H-11, H-17, H-18
13		46.6 *s*	H-16, H-18, H-20
14	1.14 *d* (5.7)	55.2 *d*	H-11, 18
15	β 1.45 *d* (7.0)	31.9 *t*	
	α 1.85 *m*		
16	4.66 *dd* (7.1, 15.7)	81.6 *d*	
17	2.25 *m*	61.8 *d*	H-18
18	1.23 *s*	10.9 *q*	
19	0.85 *s*	19.1 *q*	
20	3.21 *t* (6.9)	36.8 *d*	H-21
21	1.48 *d* (6.9)	13.7*q*	H-17, 20
22		112.5 *s*	H-20, 21, 26
23	4.10 *m*	63.1 *d*	H-24
24	eq 1.93 *m*	35.8 *t*	H-26, 27
	ax 2.25 *m*		
25	1.85 *m*	30.3 *d*	H-24
26	eq 3.31 *d* (11.0)	64.0 *t*	H-27
	ax 4.04 *dd* (2.6, 11.0)		
27	1.10 *d* (7.2)	17.4 *q*	H-25, H-26
Glucosyl			
1′	4.93 *d* (7.8)	102.5 *d*	H-4′
2′	3.98 *dd* (7.8, 8.9)	75.3*d*	H-5′
3′	3.69 *dd* (8.8, 9.4)	76.9 *d*	-
4′	4.48 *dd* (9.3, 9.4)	77.95 *d*	H-5′
5′	4.23 *m*	76.5 *d*	H-4′, H-2′
6′	4.12 *dd* (2.9, 11.9)	61.3 *t*	H-4′
	4.28 *dd* (6.2, 11.9)		
Rhamnosyl			
1″	5.92 *bs*	102.2 *d*	H-4′, H-5″
2″	4.71 *bd* (3.3)	72.4 *d*	H-4″
3″	4.60 *dd* (3.3, 9.3)	72.6 *d*	H-4″
4″	4.36 *dd* (9.3, 9.3)	73.8 *d*	H-6″
5″	5.04 *dq* (9.3, 6.2)	70.1 *d*	H-4″, H-6″
6″	1.73 *d* (6.2)	18.3 *q*	H-4″

[a] Assignments were established by DEPT, HSQC and HMBC data.
[b] 500 MHz, chemical shifts in ppm relative to TMS; *J* in Hz.
[c] 125 MHz; multiplicity was established from DEPT data.

12β-OH orientation. Likewise, the proton H-23 at δ 4.10 correlated with the protons H-24$_{eq}$ at δ 1.93, H-20 at δ 3.21 and H$_3$-27 at δ 1.10, which defined (23*S*, 25*S*) configuration. The diglycoside moiety was determined as *O*-α-rhamnopyranosyl-(1 → 4)-β-glucopyranosyl linked to the C-3-OH of the aglycone by HMBC analyses, i.e., correlations between the carbon at δ 102.2 with the proton at δ 4.48 and between the carbon at δ 77.98 with the anomeric glucosidic proton at δ 4.93. Thus, compound **3** was identified as (23*S*, 25*S*)-12β,23-dihydroxyspirost-5-en-3β-yl *O*-α-rhamnopyranosyl-(1 → 4)-β-glucopyranoside.

Table 3
DPPH radical scavenging activity of compounds **1**, **2**, **10** and **11** isolated from *Veronica turrilliana*

Compound	DPPH scavenging activity (IC_{50})[a] μg/ml (μM)
Turrillide A (**1**)	29.3 (37.2)
Turrillide B (**2**)	31.8 (33.1)
Arbutin (**10**)	74.4 (274)
6-*O*-*E*-Caffeoylarbutin (**11**)	113 (260)
Quercetin[b]	16.8 (55.6)

[a] Concentrations of compounds required to quench 50% of the free DPPH radical, determined graphically from the spectrophotometric measurements of reactions between an equal volumes of compound solution (800–0.39 μg/ml) and DPPH (80 μg/ml) in MeOH.
[b] Quercetin as positive reference.

The known C$_9$ iridoid glucosides, catalpol (**4**), catalposide (**5**), verproside (**6**), amphicoside (**7**), isovanilloylcatalpol (**8**) and aucubin (**9**) (Taskova et al., 2002), as well as the phenolics, arbutin (**10**) (Sakar et al., 1991) and 6-*O*-*E*-caffeoylarbutin (**11**) (Machida and Kikuchi, 1993) were identified spectroscopically (MS, NMR) and by comparison with reported data. Arbutin (**10**) and caffeoylarbutin (**11**) were found for the first time in genus *Veronica*. Arbutin is a diuretic, emmenagoge, and antioxidant agent and has been used in treatment of urinary tract infections (Azadbakht et al., 2004).

Evaluation of the DPPH radical scavenging potential of **1**, **2**, **10** and **11** revealed that the two phenylethanoids were more potent (approximately 1.6-fold) than the flavonoid quercetin (Table 3). The activity of **10** and **11** was much weaker indicating that the caffeoyl moiety alone does not contribute to the activity significantly (Foti et al., 2004).

The inhibitory concentration of **1**–**3**, **10** and **11** were tested on four fungal (*Botrytis cinerea*, *Cladosporium herbarum*, *Eurotium amstelodami*, *Leptosphaeria maculans*) and two bacterial strains (Gram-positive, *Bacillus subtilis* and Gram-negative, *Pseudomonas syringae*). The compounds were proven inactive against the tested microorganisms in concentrations of 0.39–800 μg/ml.

3. Experimental

3.1. General methods

UV spectra were recorded on a Heλios Gamma UV—Vis spectrophotometer. IR spectra were obtained on a Bruker IFS 113v. Optical rotations were measured on a Perkin–Elmer 241 polarimeter. ^{1}H (250 and 500 MHz) and ^{13}C NMR (62.5 and 125 MHz) spectra were recorded on Bruker DRX 250 and Bruker 500 spectrometers with TMS as an internal standard. HMBC experiments were

optimised for $^{2,3}J_{H/C} = 8$ Hz. HR-FABMS were obtained with a Micromass LCT TOF, MS/MS experiment were performed on a Bruker Daltonics Esquire 4000 system and ESI/MS with a Micromass ZQ spectrometer. LPLC was carried out with Merck Lobar RP-8 and RP-18 columns.

3.2. Plant material

Aerial parts of *V. turrilliana* were collected in Strandzha Mountain and identified by Dr. Rilka Taskova. The voucher specimen, 154 089 was deposited in the Herbarium of the Institute of Botany (SOM), Bulgarian Academy of Sciences, Sofia.

3.3. Extraction and isolation

Air dried and ground plant material (70 g) was extracted with 500 ml MeOH (2 × 48 h). The H_2O-soluble part of the MeOH extract (9.9 g) was extracted with Et_2O (3 × 200 ml). The aq. layer was concentrated and subjected to polyamide column chromatography eluting with H_2O, H_2O–MeOH mixtures (25–100%) to give eight fractions, A–H. Fr. A (1.4 g) was separated on a Lobar column (RP-8, size B, eluents 5–25% MeOH) to give **4** (18 mg) and **9** (17 mg). Compound **10** (650 mg) was purified from fr. B (2.0 g) by LPLC (Lobar RP-8, size B, eluents 5–50% MeOH). Fr. C (0.75 g) was separated on a Lobar column (RP-8, size B, eluents 5–80% MeOH) to afford **3** (115 mg) and two other fractions (81 and 71 mg) additionally purified by PTLC with $CHCl_3$–MeOH–H_2O (61:32:7) to give **6** (6 mg) and on a Lobar column (RP-18, size A, eluents 5–45%) to afford **7** (10 mg) and **8** (27 mg). Fr. D (2.0 g) was subjected to LPLC (Lobar RP-8, size B, eluents 10–28% MeOH) to afford further amount of **3** (80 mg) and a fraction (82 mg) additionally purified by PTLC with $CHCl_3$–MeOH–H_2O (61:32:7) to obtain **1** (55 mg) and **6** (15 mg). Fr. E (0.23 g) was applied on a silica gel column eluting with $CHCl_3$–MeOH–H_2O mixtures (60:15:4, 60:22:4, 61:32:7) and a fraction (58 mg) further purified by PTLC with *t*-BuOMe–MeOH–H_2O (12:2:1) to obtain **3** (28 mg) and **5** (5 mg). Frs. F (0.41 g) and H (0.45 g) were separated by silica gel chromatography at the same conditions and additionally purified on a Lobar column (RP-18, size A, eluents 10–55% MeOH) to afford **2** (34 mg) and by PTLC with EtOAc–MeOH–H_2O (63:12:9) yielding **11** (33 mg), respectively.

3.3.1. Turrillioside A (*1*)

Amorphous powder (55 mg); $[\alpha]_D^{20}$ − 7.9° (MeOH; c 0.5); UV (MeOH) λ_{max} nm (log ε):217 (4.09), 301 (3.89), 331 (4.0); IR ν_{max}^{KBr} cm^{-1} 3410, 1703, 1635, 1600, 1520; HR-FABMS found 809.2466 $[M+Na]^+$ (calculated 809.2480 for $C_{35}H_{46}O_{20}Na$); pos. ESI/MS *m/z* (rel. int.): 1595.0 (21) $[2M+Na]^+$, 808.8 (100) $[M+Na]^+$, 324.8 (8) [M-Glc-Rhm-Agl]$^+$; neg. ESI/MS: 1570.9 (18) $[2M-H]^-$, 784.8 (100) $[M-H]^-$; ^{1}H and ^{13}C NMR see Table 1.

3.3.2. Turrillioside B (*2*)

Amorphous powder (34 mg); $[\alpha]_D^{20}$ − 26.0° (MeOH; c 0.5); UV (MeOH) λ_{max} nm (log ε): 216 (3.99), 292 (3.96), 326 (2.4); IR ν_{max}^{KBr} cm^{-1} 3390, 1697, 1630, 1605, 1520; HR-FABMS found 985.2965 $[M+Na]^+$ (calculated 985.2954 for $C_{45}H_{54}O_{23}Na$); pos. ESI/MS *m/z* (rel. int.): 1947.1 (11) $[2M+Na]^+$, 984.9 (100) $[M+Na]^+$; neg. ESI/MS: 1923.1 (8) $[2M-H]^-$, 960.8 (100) $[M-H]^-$; ^{1}H and ^{13}C NMR see Table 1.

3.3.3. Turrillianoside (*3*)

White solid (223 mg); $[\alpha]_D^{20}$ − 62.6° (MeOH; c 0.43); IR ν_{max}^{KBr} cm^{-1} 3407, 2960, 2937, 2907, 2872, 2852, 1640, 1457, 1133–1041, 986, 947, 913, 839, 810 cm^{-1}; HR-FABMS, found: 755.4227 $[M+H]^+$, calculated: 755.4218 (for $C_{39}H_{63}O_{14}$); pos. ESI/MS *m/z* (rel. int.): 1531.6 (28) $[2M+Na]^+$, 777.1 (100) $[M+Na]^+$; neg. ESI/MS *m/z* (rel. int.): 1507.1 (5) $[2M-H]^-$, 753.1 (40) $[M-H]^-$; ^{1}H and ^{13}C NMR see Table 2.

3.3.4. Acid hydrolysis of compounds *1–3*

A solution of each compound (5 mg) in 2 M HCl (2 ml) was refluxed for 1 h. The reaction mixture was diluted with H_2O and then extracted with $CHCl_3$. The aq. layer was neutralized with $BaCO_3$, the precipitate filtered and the filtrate evaporated giving a residue which was identified by comparison with authentic samples of glucose ($R_f = 0.43$) and rhamnose ($R_f = 0.72$) by TLC on silica gel with *i*-PrOH–toluene–EtOAc–H_2O (20:4:10:5).

3.4. Radical scavenging activity

The photometric assay method (Blois, 1958) was modified to suit for 96-well microplate reading. Briefly, the test compounds (**1**, **2**, **10** and **11**) were deposited and serially diluted twofold across the wells to obtain a series of 12 concentrations in the range of 800–0.39 μg/ml, 100 μl per well. To each wells 100 μl of 2,2-diphenyl-1-picrylhydrazyl (DPPH, 80 μg/ml in MeOH) was added and mixed by shaking. After incubation for 30 min at room temperature the absorbance at 517 nm was recorded spectrophotometrically (OptiMax, Molecular Devices, USA). The concentrations of compounds that gave 50% scavenging potential (IC_{50}, in μg/ml) were determined graphically from the regression curve. The pure solvent and DPPH was used as the blank, and quercetin was used as a reference compound in the same concentration range as the test compounds. All the analyses were done in 4 replicates.

3.5. Antimicrobial assays

The minimum inhibitory concentration was determined in 4 replicates per test strain, compound and concentration, using a ×2 microdilution method (Kokubun et al., 2003). Malt extract broth (2%, Oxoid, Basingstoke, UK) and YEPP medium containing yeast extract (0.3%), bacteriological peptone (1%) and NaCl (0.5%) were used for fungi

and bacteria, respectively. The positive control for fungi was nystatin, and that for bacteria was chloramphenicol. The following microorganisms were used: *Botrytis cinerea* IMI160282; *Cladosporium herbarum* IMI300461; *Eurotium amstelodami* FC1021; *Leptosphaeria maculans* IMI266355; *Bacillus subtilis* IMI347329; and *Pseudomonas syringae* IMI347448 (ATCC19310).

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