Phenylethanoid Glycosides from Lantana fucata with in Vitro Anti-inflammatory Activity

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · AUGUST 2009

Impact Factor: 3.8 · DOI: 10.1021/np9002383 · Source: PubMed

Phenylethanoid Glycosides from *Lantana fucata* with *in Vitro* Anti-inflammatory Activity

Lisieux de Santana Julião,[†] Anna Lisa Piccinelli,[‡] Stefania Marzocco,[‡] Suzana Guimarães Leitão,[†] Cinzia Lotti,[‡] Giuseppina Autore,[‡] and Luca Rastrelli*[,‡]

Programa de Biotecnologia Vegetal, Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, CCS, Bl. A, 2 andar, Ilha do Fundão, 21.941-590, Rio de Janeiro, RJ, Brazil, and Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy

Received May 7, 2009

A phytochemical analysis of *Lantana fucata* dried leaves led to the isolation of three new phenylethanoid glycosides, fucatosides A−C, along with parvifloroside A and six known methoxyflavones. Their structures were established by NMR and ESIMS experiments. *In vitro* assays showed that the alcoholic extract and fucatoside C have significant anti-inflammatory effects, inhibiting NO release in the LPS-induced J774.A1 murine macrophage cell line.

Lantana (Verbenaceae) is a neotropical genus with approximately 150 species, many of them occurring in Brazil. *L. fucata* Lindl. (*L. lilacina* Desf.) is a small shrub that produces pink or purple flowers and is found in tropical and subtropical temperate regions in the Americas. It is known as a weed and an ornamental plant,[1] and the leaves have been used in Brazilian traditional medicine as a carminative and anti-inflammatory and to treat colds and bronchitis as infusions, decoctions, and tinctures.[1−3] Nowadays leaves of *L. fucata* can be found in the local market as dried powder, tincture, alcoholic, and aqueous-alcoholic preparations. However, apart from a previous study that reported the occurrence of an acyclic monoterpene ester glucoside and verbascoside in the leaves,[3,4] there are no literature data concerning the chemical composition and pharmacological properties of *L. fucata*.

A phytochemical analysis of the EtOAc extract of *L. fucata* leaves led to the isolation of phenylethanoid glycosides and methoxyflavones. The structures of these compounds were elucidated by extensive spectroscopic methods including 1D and 2D NMR experiments as well as ESIMS analysis.

The anti-inflammatory activity of the isolated phenylethanoid glycosides and the traditional aqueous, alcoholic, and aqueous-alcoholic (70%) extracts of the leaves was determined in a model of *Escherichia coli* lipopolysaccharide (LPS)-induced nitric oxide (NO) release in the J774A.1 murine macrophage cell line. Alcoholic extracts were obtained by macerating the dried leaves in alcoholic solutions of 96% (v/v) and 70% (v/v) EtOH to obtain 1 L of tincture, decoction, and infusion of leaves in boiling H_2O, according to the traditional preparations.

Results and Discussion

The dried leaves of *L. fucata* were exhaustively extracted with EtOH to afford a brown syrup. This dried residue was partitioned between H_2O and organic solvents of increasing polarities, to afford *n*-hexane, CH_2Cl_2, and EtOAc extracts. Extraction with low-polarity solvents yielded more lipophilic components, while EtOAc afforded a larger spectrum of nonpolar and polar material. Extractions with *n*-hexane and CH_2Cl_2 were therefore used as a prepurification step to selectively remove interfering components. The dried EtOAc extract was fractionated by gel filtration on a Sephadex LH-20 column and by RP-HPLC, giving three new phenylethanoid glycosides (**1**−**3**), together with parvifloroside A (**4**)[5] and six methoxyflavones (**5**−**10**), identified as 4′-*O*-methylscutellarein (**5**),[6] nepetin (**6**),[7] 5,3′,4′-trihydroxy-6,7,5′-trimethoxyflavone (**7**),[8] cirsiliol (**8**),[8] salvigenin (**9**),[9] and 5,3′,5′-trihydroxy-6,7,4′-trimethoxyflavone (**10**),[10] by comparison of their spectroscopic data, especially NMR, with those in the literature. (Figures 1 and 2).

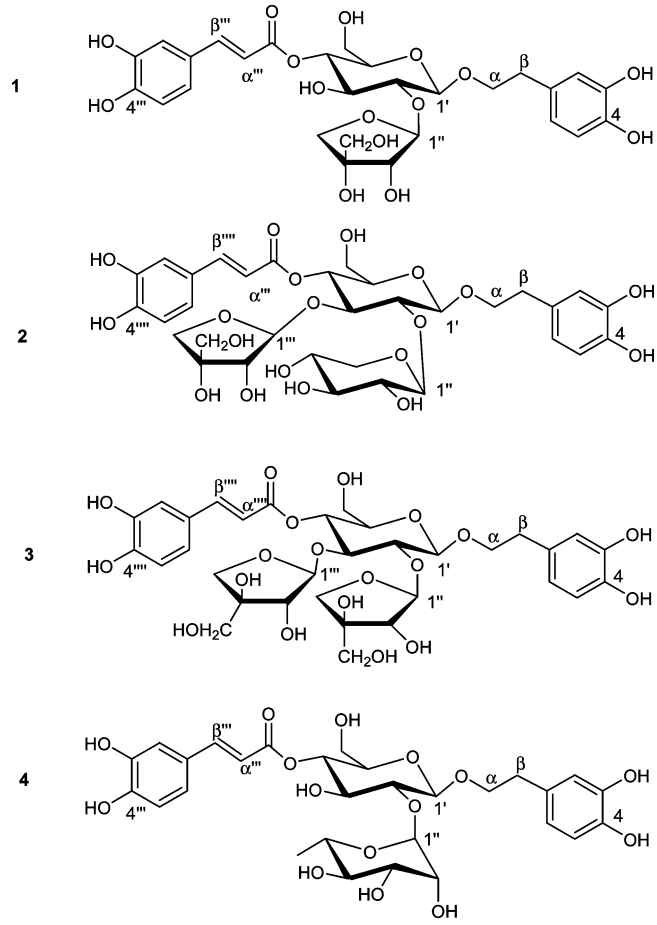

Figure 1. Phenylethanoid glycosides **1**−**4** isolated from *L. fucata* leaves.

The HRESIMS of **1** gave an $[M - H]^-$ ion at *m/z* 609.1800, indicating a molecular formula of $C_{28}H_{33}O_{15}$ and in good agreement with the observation of five methylene, 15 methine, and eight quaternary carbon resonances in its HSQC and HMBC NMR spectra (Table 1). The negative ESIMS spectrum showed a deprotonated molecule $[M - H]^-$ at *m/z* 609, and ESIMSMS experiments showed further fragment ions at *m/z* 447 $[M - H - 162]^-$, *m/z* 315 $[M - H - 162 - 132]^-$, and *m/z* 297 $[M - H - 162 - 132 - 18]^-$, suggesting the successive loss of a caffeoyl unit, a pentosyl moiety,

* To whom correspondence should be addressed. Tel: 0039 89 969766. Fax: 0039 89 969602. E-mail: rastrelli@unisa.it.
[†] Universidade Federal do Rio de Janeiro.
[‡] University of Salerno.

10.1021/np9002383 CCC: $40.75
Published on Web 07/27/2009

Table 1. ^{1}H NMR and ^{13}C NMR Data and HMBC Correlations of Compounds **1** and **4** in Methanol-d_4[a] (600 MHz)

	1				**4**		
position	δ_H (J_{HH} in Hz)	δ_C	HMBC	position	δ_H (J_{HH} in Hz)	δ_C	HMBC
aglycone				*aglycon*			
1		131.1		1		130.6	
2	6.79, d (1.5)	116.5	3	2	6.72, d (1.5)	116.5	6
3		144.5		3		144.0	
4		146.0		4		145.3	
5	6.70, d (7.4)	116.8	3, 4	5	6.70, d (7.4)	116.8	1, 4
6	6.61, dd (7.4, 1.5)	120.8	5	6	6.59, dd (7.4, 1.5)	121.2	3, 5
α	3.66, 4.08, m	72.4		α	3.75, 4.08, m	72.3	
β	2.83, m	36.1	α, 1, 2	*β*	2.81, m	36.3	α, 1, 2
glucose				*glucose*			
1′	4.41, d (7.4)	104.0		1″	4.41, d (7.9)	104.1	α, 2″
2′	3.81, dd (9.2, 7.4)	81.6		2″	3.84, dd (9.5, 7.9)	81.7	
3′	3.78, t (9.2)	75.5		3″	3.57, t (9.5)	75.3	
4′	4.91, t (9.2)	70.4		4″	4.94, t (9.5)	70.2	3″, 5″, 6″, CO
5′	3.50, m	76.3		5″	3.40, m	76.1	1″
6′a	3.62, dd (12.0, 5.0)	62.2		6″	3.56, dd (12.0, 5.0)	62.0	
6′b	3.75, dd (12.0, 3.5)				3.65, dd (12.0, 3.5)		
apiose				*rhamnose*			
1″	5.37, d (3.5)	111.2	2′, 2″	1‴	5.22, d (1.5)	102.9	2″, 2‴, 5‴
2″	3.92, d (3.5)	78.3		2‴	3.95, dd (3.0, 1.5)	72.3	
3″		80.2		3‴	3.32, dd (9.5, 3.0)	73.6	
4″a	4.02, d (11.2)	76.0		4‴	3.60, t (9.5, 9.5)	71.0	
4″b	4.15, d (11.2)						
5″a	3.63, d (9.3)	65.8	1″	5‴ 6‴	4.05, dq (9.5, 6.1)	69.8	5‴, 3‴
5″b	3.49, d (9.3)				1.12, d (6.1)	18.2	
caffeoyl				*caffeoyl*			
1‴		123.8		1′		127.2	
2‴	7.08, d (1.5)	115.1	*β*‴, 1‴, 3‴	2′	7.08, d (1.5)	115.3	*β*′, 3′, 6′
3‴		151.1		3′		146.6	
4‴		147.2		4′		146.4	
5‴	6.82, d (8.2)	117.1	3‴, 4‴, 6‴	5′	6.80, d (7.9)	116.5	4′, 1′
6‴	6.99, dd (8.2, 1.5)	123.2	*β*‴, 2‴	6′	6.98, dd (7.9, 15)	123.1	α′, *β*′, 4′
α‴	6.31, d (15.4)	114.8	1‴, CO	α′	6.30, d (15.8)	117.6	1′
β‴	7.63, d (15.4)	147.0	α‴, 6‴, CO	*β*′	7.62, d (15.8)	148.0	α′, 6′, CO
CO		168.0		CO		167.7	

[a] *J* values are in parentheses and reported in Hz; assignments by DQF- COSY, 1D TOCSY, HSQC, and HMBC experiments.

and water. The ^{1}H NMR spectrum of **1** (see Table 1) exhibited the characteristic signals belonging to (*E*)-caffeic acid and 3,4-dihydroxyphenylethanol moieties: protons of aromatic rings (2 × ABX systems), two *trans*-olefinic protons (AB system, $J_{AB} = 15.4$ Hz), *β*-methylene at *δ* 2.83 (2H, m), and two diastereotopic protons at *δ* 4.08 and 3.66 (each 1H, m) of the side-chain of the aglycon moiety. Additionally, two anomeric proton resonances appeared at *δ* 5.37 ($J = 3.5$ Hz) and 4.41 ($J = 7.4$ Hz). 1D-TOCSY, DQF-COSY, and HSQC NMR experiments showed the presence of one *β*-apiofuranosyl unit and one *β*-glucopyranosyl unit in the structures of **1**. The apiose moiety was confirmed by the observation of three pairs of ABq signals at *δ* 5.37 and 3.92 ($J = 3.5$ Hz), 4.15 and 4.02 ($J = 11.2$ Hz), and 3.63 and 3.49 ($J = 9.3$ Hz) in the ^{1}H NMR. The HSQC spectrum showed signals for CH_2 at *δ* 76.03 and 65.84 and lack of a signal at *δ* 80.16. Analysis of HMBC correlations and ROE measurements showed that these elements were best arranged as a *β*-D-erythroapiofuranose.[11] The configurations of the sugar moieties were assigned after hydrolysis of **1** with 1 N HCl. The hydrolysate was dissolved in 1-(trimethylsilyl)imidazole and pyridine (0.1 mL), and GC retention times of each sugar as TMS-imidazolyl derivatives were compared with those of authentic samples prepared in the same manner. In this way, the sugar units of **1** were determined to be D-apiose and D-glucose. The caffeoyl group was positioned at C-4′ of the glucose on the basis of the strong deshielding of H-4′ of the glucose unit (*δ* 4.91, t, $J = 9.2$ Hz). The HSQC spectrum also showed glycosylation shifts for C-2 (*δ* 81.62), suggesting that the *β*-apiofuranosyl was a terminal unit. These results indicated that the structure of **1** is closely related to that of parvifloroside A (**4**). An unambiguous determination of the sequence and linkage sites was obtained from the HMBC correlations. Thus, cross-peaks were observed between the anomeric proton of glucose (*δ* 4.41, H-1′) and C-α (*δ* 72.41) of the phenylethyl alcohol moiety, H-4′ (*δ* 4.91) of glucose and the carbonyl carbon resonance (*δ* 168.00) of the acyl moiety, and H-2′ of glucose (*δ* 3.81) and the anomeric carbon of the apiose unit (*δ* 111.20, C-1″) Therefore, the structure of **1** was established as 1-*O*-3,4-(dihydroxyphenyl)ethyl-*β*-D-apiofuranosyl-(1→2)-4-*O*-caffeoyl-*β*-D-glucopyranoside, named fucatoside A.

Compound **2** showed a major ion peak at *m/z* 741 $[M - H]^-$ in the negative ESIMS and significant fragment ion peaks at *m/z* 579 $[M - H - 162]^-$, *m/z* 447 $[M - H - 162 - 132]^-$, and *m/z* 315 $[M - H - 162 - 132 - 132]^-$ in the ESIMS/MS experiments, suggesting the successive loss of a caffeoyl unit and two pentosyl moieties. In conjunction with the analysis of the HSQC and HMBC NMR data, its molecular formula was deduced to be $C_{33}H_{42}O_{19}$ by HRESIMS.

Compound **2** showed similar 1D and 2D NMR features to those of compound **1** except for the presence of a third sugar unit assigned to a *β*-xylose, characterized by large *trans*-diaxial proton coupling constants and by 1,3- and 1,5-diaxial relationships in the ROESY spectrum. Analysis of 2D NMR experiments (COSY and HSQC) showed that this compound contained a *β*-glucose unit, as in compound **1**, whose deshielded C-3 resonance (*δ* 83.44) indicated a substitution by a second pentosyl unit. Acidic hydrolysis afforded monosaccharide components identified as D-glucose, D-xylose, and D-apiose by GC analysis. Finally, all connectivities within **2** were proven by an HMBC experiment, where correlations between H-1′ (*δ* 4.53) of the glucose unit and the α-C atom (*δ* 72.14) of the phenethyl moiety, H-1″ (*δ* 4.62) of the xylose and C-2′ (*δ* 82.50) of the glucose unit, H-1‴ (*δ* 5.33) of the apiose and C-3′ (*δ* 83.44) of the glucose unit, and H-4′ (*δ* 4.93) of the glucose and the carbonyl carbon (*δ* 169.00) of the caffeoyl moiety were observed (Table 2). Consequently, the structure of compound **2** was established as 1-*O*-3,4-(dihydroxyphenyl)ethyl-*β*-D-apiofuranosyl-(1→2)-

Table 2. ^{1}H and ^{13}C NMR Data and HMBC Correlations of Compounds **2** and **3** in Methanol-d_4[a] (600 MHz)

	2				**3**		
position	δ_H (J_{HH} in Hz)	δ_C	HMBC	position	δ_H (J_{HH} in Hz)	δ_C	HMBC
aglycone				*aglycon*			
1		132.8		1		131.3	
2	6.73, d (1.7)	117.3	3, 6	2	6.70, d (1.7)	116.6	3, 6
3		147.4		3		145.7	
4		145.6		4		144.3	
5	6.70, d (7.9)	117.1	1, 4	5	6.67, d (8.3)	116.6	1, 4
6	6.61, dd (7.9, 1.7)	122.0	4, 5	6	6.60, d (8.3, 1.7)	121.0	4, 5
α	3.73, 4.08, m	72.1	1, 1′	α	3.74, 4.06, m	71.5	
β	2.81, m	36.1	α, 1, 2, 3	*β*	2.82, m	36.1	α, 1, 2, 6
glucose				*glucose*			
1′	4.53, d (7.6)	103.4	α, 2′	1′	4.46, d (7.4)	102.5	α, 2′
2′	3.66, dd (9.4, 7.6)	82.5	1′, 3′	2′	3.53, dd (9.3, 7.4)	79.4	
3′	3.91, t (9.4)	83.4	1′, 2′, 4′	3′	3.84, t (9.3)	82.8	2′, 4′, 1″
4′	4.93, t (9.4)	71.2	3′, 5′, 6′, CO	4′	4.93, t (9.3)	70.0	3′, 5′, CO
5′	3.59, m	75.9		5′	3.53, m	75.6	
6′a	3.59, dd (12.0, 5.0)	62.7		6′a	3.54, dd (12.0, 5.0)	61.8	
6′b	3.74, dd (12.0, 3.8)			6′b	3.64, dd (12.0, 3.5)		
xylose				*apiose*			
1″	4.62, d (7.4)	105.7	2′	1″	5.22, d (1.2)	111.9	
2″	3.25, dd (7.4, 8.9)	76.0	3″	2″	3.90, d (1.2)	78.1	
3″	3.36, dd (8.9, 8.9)	77.8	2″, 4″	3″		79.9	
4″	3.56, m	71.3	2″	4″a	3.73, d (9.6)	75.0	1″, 2″, 3″
				4″b	3.97, d (9.6)		
5″a	3.19, dd (11.0, 2.5)	67.4	1″, 2″	5″a	3.51, d (12.1),	64.7	1″, 4″
5″b	3.90, dd (11.0, 5.0)			5″b	3.66, d 12.1)		
apiose				*apiose*			
1‴	5.33, d (3.2)	112.2	3′, 2‴, 4‴	1‴	5.33, d (1.2)	111.5	
2‴	3.97, d (3.2)	78.7	1‴, 5‴	2‴	3.94, d (1.2)	77.8	
3‴		81.6		3‴		80.0	
3‴		81.6		3‴		80.0	
4‴a	3.76, d (9.8)	75.0	1‴, 3‴, 5‴	4‴	3.78, d (9.6)	74.7	1‴, 2‴, 3‴
4‴b	4.11, d (9.8)				4.01, d (9.6)		
5″	3.52, br s	65.5	1‴, 3‴, 4‴	5‴a	3.49, d (12.1)	65.3	
				5‴b	3.69, d (12.1)		
caffeoyl				*caffeoyl*			
1⁗		123.9		1′		127.2	
2⁗	7.07, d (2.0)	115.4	1⁗, 3⁗, 4⁗	2′	7.05,d (1.5)	114.8	1⁗, 3⁗
3⁗		149.7		3′		149.3	
4⁗		147.4		4′		146.1	
5⁗	6.80, d (8.2)	117.0	3′, 4′, 6′	5′	6.78, d (7.5)	116.3	3′, 4′, 6′
6⁗	6.97, dd (8.2, 2.0)	123.0	*β*′, 2′, 4′	6′	6.95, dd (7.5, 1.5)	122.6	*β*′, 2′
α⁗	6.30, d (15.9)	116.0	1′, CO	α′	6.30, d (15.8)	114.8	1′, CO
β⁗	7.61, d (15.9)	148.3	1′, 2′, 6′, CO	*β*′	7.61, d (15.8)	147.3	1′, 2′, CO
CO		169.0		CO		168.2	

[a] *J* values are in parentheses and reported in Hz; assignments by DQF-COSY, 1D TOCSY, HSQC, and HMBC experiments.

β-D-xylopyranosyl-(1→3)-4-*O*-caffeoyl-*β*-D-glucopyranoside, named fucatoside B.

The ESIMS in negative mode of compound **3** exhibited a quasi-molecular ion peak at *m/z* 741 $[M - H]^-$ and significant fragment ion peaks at *m/z* 579 $[M - H - 162]^-$, *m/z* 447 $[M - H - 162 - 132]^-$, and *m/z* 315 $[M - H - 162 - 132 - 132]^-$ in ESIMSMS experiments, suggesting, as for fucatoside B (**2**), the successive loss of a caffeoyl unit and two pentosyl moieties. A high-resolution measurement indicated the molecular formula, $C_{33}H_{42}O_{19}$, in accordance with HSQC and HMBC NMR analysis.

Analysis of the ^{1}H NMR spectrum of compound **3** in comparison to that of **2** showed that the difference between the two compounds should be confined to the sugar portion. Three doublets of anomeric protons at *δ* 4.46, 5.22, and 5.33 indicated its trisaccharide nature. Analysis of 2D NMR data (COSY, TOCSY, and HSQC) showed that this compound contained a *β*-glucose unit. In the COSY experiments, the anomeric protons at *δ* 5.22 and 5.33 (both $J = 1.2$ Hz) were observed to couple with vicinal protons at *δ* 3.90 and 3.94, respectively, also doublets (both $J = 1.2$ Hz), and assigned to H-2. These sugars were further characterized in the ^{1}H NMR spectra by two other pairs of doublets corresponding to hydroxymethylene groups with attached carbons at *δ* 74.99 and 64.66 and at *δ* 74.68 and 65.28, respectively (Table 2). Analysis of HMBC correlations and ROE measurements showed that these elements were best arranged as two α-threo-apiofuranoside moieties with quaternary carbons at *δ* 79.95 and 79.99.[11] Indeed, acid hydrolysis of **3** afforded D-glucose and L-apiose in the ratio 1:2. All other signals in the ^{1}H NMR and ^{13}C NMR spectra of **3** were assigned after analysis of the 2D NMR data (Table 2). The carbon resonances assigned to the α-apiose units showed no unusual chemical shifts, suggesting their terminal position. HMBC experiments established the binding site of the two apiose units at C-2 (H-1″/C-2′) and C-3 (H-1‴/C-3′) and the caffeoyl unit at C-4 of the glucose (H-4′/CO).

On the basis of these data, compound **3** was assigned the structure 1-*O*-3,4-(dihydroxyphenyl)ethyl-*O*-α-L-apiofuranosyl-(1→2)-*O*-α-L-apiofuranosyl-(1→3)-4-*O*-caffeoyl-*β*-D-glucopyranoside, named fucatoside C.

Another phenethyl alcohol glycoside, 1-*O*-3,4-(dihydroxyphenyl)ethyl-α-L-rhamnopiranosyl-(1→2)-4-*O*-caffeoyl-*β*-D-glucopyranoside (**4**), was also isolated from *L. fucata*. Its structure was shown to be identical with parvifloroside A, reported recently from *Stachys parviflora*,[5] and this appears to be the second report of this compound. Known methoxyflavones **5**−**10** were also isolated from *L. fucata*. Their structures were elucidated by ESIMS and ^{1}H and ^{13}C NMR data analysis.

It is noteworthy that there are very few examples of phenylethanoid glycosides with a trisaccharide moiety containing apiose. Additionally, fucatosides A−C and parvifloroside A are closely

Table 3. Effect of *L. fucata* Extracts[a] on Nitrite Release, Index of NO Production, by J774A.1 Macrophages Activated with *E. coli* Lipopolysaccharide (LPS)[b]

	nitrite release inhibition (% vs LPS)		
extract	100 μg/mL	10 μg/mL	1 μg/mL
LFHA	95.6 ± 1.5***	74.4 ± 2.6***	13.4 ± 0.95
LFA	92.4 ± 2.9***	85.9 ± 3.0***	35.2 ± 0.9**
LFD	98.9 ± 3.4***	17.5 ± 2.2	0.1 ± 0.02
LFI	13.26 ± 0.5***	13.4 ± 1.7	0.3 ± 0.3

[a] LFA: alcoholic tincture, LFHA: 70% aqueous-alcoholic tincture, LFD: decoction, LFI: infusion of *L. fucata*. [b] Data are expressed as means ± SEM of percentage inhibition vs nitrite release by J774A.1 macrophage treated with LPS alone. *** and ** denote $P < 0.001$ and $P < 0.01$, respectively, vs LPS alone.

Table 4. Effect of Phenylethanoid Glycosides on Nitrite Release by J774A.1 Macrophages Stimulated with *E. coli* Lipopolysaccharide (LPS)[a]

	nitrite release inhibition (% vs LPS)		
compound	100 μg/mL	10 μg/mL	1 μg/mL
fucatoside A (**1**)	52.5 ± 1.9***	11.2 ± 0.9	13.3 ± 0.6
fucatoside B (**2**)	81.4 ± 3.9***	16.41 ± 2.0	13.7 ± 0.89
fucatoside C (**3**)	93.7 ± 2.5***	31.8 ± 1.2***	4.4 ± 0.6
parvifloroside A (**4**)	88.5 ± 2.4***	9.0 ± 1.6	3.8 ± 0.15

[a] Data are expressed as inhibition percentage means ± SEM vs nitrite production in 24 h by J774A.1 macrophages treated with LPS alone. *** denotes $P < 0.001$ vs LPS-treated macrophages.

related to verbascoside previously isolated from this species.[3,4] The pharmacological activities of phenylethanoids have been extensively reviewed.[12] In particular they have been shown to possess anti-inflammatory activity,[13] which led us to investigate the *in vitro* activities of traditional aqueous extracts, obtained as decoction (LFD) and infusion (LFI), alcoholic and aqueous-alcoholic extracts (LFA and LFHA, respectively), and isolated phenylethanoid glycosides **1**−**4** on inducible nitric oxide synthase (iNOS) activity, evaluating nitrite production, index of nitric oxide biosynthesis, in the medium of LPS-activated J744.A1 macrophage. NO release in the cellular medium of LPS-stimulated J774.A1 macrophages, incubated with *L. fucata* extracts (1, 10, and 100 μg/mL) and phenylethanoids **1**−**4** (1, 10, and 100 μg/mL), was evaluated 24 h after LPS (6×10^3 u/mL) challenge. Results were expressed as percent inhibition evaluated versus macrophages treated with LPS alone.

Among the tested extracts only the alcoholic one, added 1 h before and simultaneously with LPS, significantly inhibited ($P < 0.01$ vs LPS) NO release in a concentration-related manner (92.4 ± 2.9, 85.9 ± 3.0, and 35.2 ± 0.9 for 100, 10, and 1 μg/mL. respectively, vs LPS alone; Table 3). Among *L. fucata* phenylethanoid constituents only fucatoside C (**3**), added 1 h before and simultaneously with LPS, significantly inhibited nitrite release in a concentration-related manner ($P < 0.001$ vs LPS) for the concentrations of 10 and 100 μg/mL, while fucatosides A and B (**1** and **2**) and parvifloroside A (**4**) significantly inhibited nitrite production release only at the highest concentration tested (100 μg/mL; Table 4).

To establish the effects of *L. fucata* preparations (LFD, LFI, LFA, and LFHA) and phenylethanoid constituents on cell viability *in vitro* (1, 10, and 100 μg/mL), extracts and compounds **1**−**4** were tested on a murine macrophage cell line (J774.A1), murine fibrosarcoma cells (WEHI-164), and human embryonic kidney cells (HEK-293) using the MTT assay.[14,15] None of the extracts (data not shown) and compounds tested showed antiproliferative activity on J774.A1 cells (Table 5).

Table 5. *In Vitro* Antiproliferative Activity of Phenylethanoid Glycosides[a]

	nitrite release inhibition (% vs LPS)		
compound	100 μg/mL	10 μg/mL	1 μg/mL
fucatoside A (**1**)	95. ± 1.9***	95.9 ± 2.9	97.3 ± 2.6
fucatoside B (**2**)	94.4 ± 2.7	96.1 ± 3.0	97.5 ± 3.5
fucatoside C (**3**)	94.3 ± 2.9	96.8 ± 2.8	96.4 ± 2.6
parvifloroside A (**4**)	96.9 ± 3.0	95.9 ± 1.9	96.7 ± 3.2
6-mercaptopurine	31.8 ± 2.8	38.0 ± 3.0	44.1 ± 1.9

[a] Data are expressed as cell viability % means ± SEM vs untreated J774A.1 macrophages.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Jasco DIP-1000 polarimeter equipped with a sodium lamp (589 nm) and a 10 cm microcell. UV spectra were obtained with a Beckman DU 670 spectrophotometer in MeOH ($c = 1$). A Bruker DRX-600 NMR spectrometer, operating at 599.19 MHz for ^{1}H and at 150.86 MHz for ^{13}C, using the UXNMR software package, was used for NMR experiments; chemical shifts are expressed in δ (parts per million) referring to the solvent peaks δ_H 3.34 and δ_C 49.0 for methanol-d_4, and coupling constants, J, are in hertz. ^{1}H−^{1}H DQF-COSY, ^{1}H−^{13}C HSQC, HMBC, and ROESY experiments were obtained by employing the conventional pulse sequences. The selective excitation spectra, 1D TOCSY, were acquired using waveform generator-based GAUSS-shaped pulses, mixing time ranging from 100 to 120 ms and a MLEV-17 spin-lock field of 10 kHz preceded by a 2.5 ms trim pulse NMR experiment. ESIMS was performed using a Finnigan LC-Q Advantage instrument from Thermoquest (San Jose, CA) equipped with Excalibur software. Exact masses were measured by an ESI/Q-TOF (Waters) instrument. Column chromatography was performed over Sephadex LH-20 (Pharmacia), and HPLC separations were carried out on a Waters 590 system equipped with a Waters R401 refractive index detector, a Phenomenex C-18 column, 10 μm (10 × 250 mm), and a U6K injector, and on an Agilent 1100 series chromatograph, equipped with a G-1312 binary pump, a G-1328A Rheodyne injector, a G-1322A degasser, and a G-1315A photodiode array detector (Waters Corp., Milford, MA) using a Waters C-18 column, 5 μm (3.9 × 150 mm, flow rate 1 mL/min). TLC analyses were performed with Macherey-Nagel precoated silica gel 60 F_{254} plates.

Plant Material. Leaves of *L. fucata* were collected in Juiz de Fora, Minas Gerais State, Brazil, in September 2005, and identified by Prof. Dr. F. R. G. Salimena, from Universidade Federal de Juiz de Fora, Minas Gerais, Brazil. A specimen of the plant (voucher no. CESJ 48653) used in this study has been deposited at the Herbarium of the same Federal University.

Extraction and Isolation. Dried and pulverized leaves (620 g) were exhaustively extracted with EtOH, and the extract was concentrated under reduced pressure to afford a brown syrup (90 g). This residue was partitioned between H_2O and organic solvents of increasing polarities, to afford the new *n*-hexane, CH_2Cl_2, and EtOAc extracts (9.4 g). Part of the EtOAc extract (6 g) was submitted to column chromatography over Sephadex LH-20 (75 × 3.5 cm) using MeOH as mobile phase at a flow rate of 2 mL/min. Sixty eight fractions of 8 mL were obtained and combined into seven fractions. Fraction 3 (70 mg) was submitted to HPLC using MeOH/H_2O (40:60) as eluent in a Phenomenex C-18 column, 10 μm (10 × 250 mm), at a flow rate of 2.5 mL/min. The major peaks (30 mg) were combined and resubjected to HPLC using MeOH/H_2O−0.05% TFA (25:75) as eluent using a Waters C-18 column, 5 μm (3.9 × 150 mm, flow rate 1 mL/min), to yield pure compounds **1** (12 mg, t_R 18.27 min), **2** (4.2 mg, t_R 22.48 min), **3** (5.0 mg, t_R 31.62 min), and **4** (4.0 mg, t_R 26.48 min). Fraction 6 (380 mg) was submitted to HPLC using MeOH/H_2O (60:40) as eluent on a Phenomenex C-18 column, 10 μm (10 × 250 mm), at a flow rate of 2.5 mL/min, to yield pure compounds **5** (1.2 mg, t_R 10.20 min), **6** (2 mg, t_R 15.5 min), **7** and **8** (1.5 mg, t_R 20.25 min), **9** (1.0 mg, t_R 23.0 min), and **10** (0.5 mg, t_R 29.1 min).

Fucatoside A (1): amorphous powder; $[\alpha]^{25}_D$ −21.32 (c 0.34, MeOH); UV λ_{max} 325, 300; ^{1}H NMR data (methanol-d_4, 600 MHz), see Table 1; ^{13}C NMR data (CD_3OD, 600 MHz), see Table 1; (−)-HRESIMS, m/z 609.1800 [M − H]$^-$, calcd for $C_{28}H_{33}O_{15}$, 609.1819; ESIMS (negative mode) m/z 609 [M − H]$^-$, MS/MS m/z 447, 315, and 297.

Fucatoside B (2): amorphous powder; $[\alpha]^{25}_D$ +0.03 (c 0.23, MeOH); UV λ_{max} 330; ^{1}H NMR data (methanol-d_4, 600 MHz), see Table 2; ^{13}C NMR data (CD_3OD, 600 MHz), see Table 2; (−)-HRESIMS, m/z 741.2252 [M − H]$^-$, calcd for $C_{33}H_{41}O_{19}$, 741.2242; ESIMS (negative mode) m/z 741 [M − H]$^-$, MS/MS m/z 579, 447, and 315.

Fucatoside C (3): amorphous powder; $[\alpha]^{25}_{D}$ +5.00 (*c* 0.15, MeOH;); UV λ_{max} 330; ^{1}H NMR data (methanol-d_4, 600 MHz), see Table 2; ^{13}C NMR data (CD_3OD, 600 MHz), see Table 2; (−)-HRESIMS, *m/z* 741.2257 $[M - H]^-$, calcd for $C_{33}H_{41}O_{19}$, 741.2242; ESIMS (negative mode) *m/z* 741 $[M - H]^-$, MS/MS *m/z* 579, 447, and 315.

Determination of the Absolute Configuration of Sugars. A solution of compounds **1**−**3** (1 mg) in 1 N HCl (0.25 mL) was separately stirred at 80 °C for 4 h. On cooling, the solution was concentrated in a stream of N_2. The residue was dissolved in 1-(trimethylsilyl)imidazole (Trisil-Z) and pyridine (0.1 mL), and the solution was stirred at 60 °C for 5 min. After drying in a stream of N_2, the residue was partitioned between H_2O and CH_2Cl_2 (1 mL, 1:1). The CH_2Cl_2 layer was analyzed by GC (Alltech l-Chirasil-Val column, 0.32 mm × 25 m; temperatures for injector and detector, 200 °C; temperature gradient system for the oven, 100 °C for 1 min and then raised to 180 °C; rate 5 °C/min). Peaks of the hydrolysate of **1**−**3** were detected by comparison with retention times of authentic samples of D- and L-apiose (t_R 19.25 and 17.15 min), D-glucose (t_R 29.11 min), and D-xylose (t_R 24.88 min) (Sigma Aldrich, St. Louis, MO) after being treated simultaneously with Trisil-Z.

Herbal Preparations. To prepare the decoction of *L. fucata* leaves, 5 g of dried leaves was boiled with 100 mL of hot H_2O for 5 min. The decoctions were then lyophilized to obtain a solid residue. The infusions were obtained by pouring 100 mL of boiling distilled water on 5 g of dried loose leaves and steeping it for 3 min. The infusions were filtered through filter paper, and the resulting infusion was freeze-dried. Extracts were obtained according to the European Pharmacopoeia.[16] Two-hundred grams of dried plant material was macerated. The alcoholic solutions used for *Lantana* preparations were 96% (v/v) and 70% (v/v) to obtain 1000 mL of tincture; the extracts were concentrated under reduced pressure to afford a brown syrup.

Cell Lines and Reagents for Bioactivity Assays. The murine macrophage cell line (J774A.1), the murine fibrosarcoma cells (WEHI-164), and the human embryonic kidney cells (HEK-293) were obtained from American Tissue Culture Collection (ATCC). *E. coli* lipopolysaccharide (LPS) was obtained from Fluka (Milan, Italy). 3-(4,5-Dimethylthiazolyl-2-yl)2,5-diphenyltetrazolium bromide (MTT) and phosphate buffer solution (PBS) were obtained from Sigma Chemical Co. (Milan, Italy). Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin, HEPES, glutamine, fetal calf serum (FCS), and horse serum were from HyClone (Euroclone-Cellbio, Pero, Milan, Italy). J774.A1 cells were grown in adhesion on Petri dishes and maintained at 37 °C as previously described.[17] WEHI-164 and HEK-293 were maintained in adhesion on Petri dishes with DMEM supplemented with 10% heat-inactivated FCS, 25 mM HEPES, 100 IU/mL penicillin, and 100 μg/mL streptomycin.

Antiproliferative Activity. J774A.1, WEHI-164, and HEK-293 (3.5×10^4 cells/well) were plated on 96-well microtiter plates and allowed to adhere at 37 °C in a 5% CO_2 atmosphere for 2 h. Thereafter, the medium was replaced with fresh medium, and serial dilutions of each test compound were added and then the cells incubated for 72 h. In some experiments, serial dilutions of 6-mercaptopurine, as reference drug, were added. Mitochondrial respiration, an indicator of cell viability, was assessed by the mitochondrial-dependent reduction of [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2*H*-tetrazolium bromide] (MTT) to formazan, and cell viability was assessed according to the method of Mosmann. Briefly, 5 μL of MTT (5 mg/mL) was added, and the cells were incubated for an additional 3 h. Thereafter cells were lysed and the dark blue crystals solubilized with 100 μL of a solution containing 50% (v/v) *N*,*N*-dimethylformamide and 20% (w/v) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter. The viability of each cell line in response to treatment with tested compounds and 6-mercaptopurine was calculated as % dead cells = 100 − (OD treated/OD control) × 100. To exclude the capacity of our tested compounds **1**−**4** to interfere with the MTT reduction assay, we performed a control experiment testing **1**−**4** without cells; they did not interfere in the reduction of MTT to formazan (data not shown).

Analysis of Nitrite. J774A.1 cells (5.0×10^4 cells/well) were plated on 96-well microtiter plates and allowed to adhere at 37 °C in a 5% CO_2 atmosphere for 2 h. Examined extracts and compounds (1−100 mg/mL for the extracts and 1−100 μg/mL for compounds) were added 1 h before and simultaneously to LPS (6×10^3 U/mL), used to induce inducible iNOS. Nitric oxide release, evaluated as nitrite (NO_2^-) accumulation in the cell culture medium, was performed 24 h after LPS stimulation by the Griess reagent.[18] The amount of nitrite in the samples was calculated from a sodium nitrite standard curve freshly prepared in culture medium. Results are expressed as percentages of inhibition calculated versus cells treated with LPS alone. *N*(G)-Nitro-L-arginine methyl ester (L-NAME; 1 μM) has been used as reference drug, able to inhibit nitrite production by LPS-treated macrophages, giving rise to 46.56 ± 1.25% inhibition of nitrite release versus LPS alone (data not shown in Table 4).

Acknowledgment. This work was supported by CNPq and FAPERJ for financial aid and CAPES for a fellowship to L.d.S.J. We thank Dr. F. R. Gonçalves-Salimena, Universidade Federal de Juiz de Fora, Minas Gerais, Brazil, for plant identification.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Lorenzi, H. In *Plantas Daninhas do Brasil*; Terrestres, Aquaticas, Parasitas e Toxicas, 3rd ed.; Instituto Plantarum de Estudos da Flora: LTDA Nova Odessa, 2000; p 540.
(2) Correa, M. P. In *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas*; Ministério da Agricultura: Rio do Jaineiro, 1984; Vol. 1, p 747.
(3) Takeda, Y.; Takechi, A.; Masuda, T.; Otsuka, H. *Planta Med.* **1998**, *64*, 78–79.
(4) Pereira, A. C.; Carvalho, H. W. P.; Silva, G. H.; Oliveira, D. F.; Figueiredo, H. C. P.; Cavalheiro, A. J.; Carvalho, D. A. *Rev. Bras. Pharmacogn.* **2008**, *18*, 204–208.
(5) Ahmad, V. U.; Arshad, S.; Bader, S.; Ahmed, A.; Iqbal, S.; Tareen, R. B. *J. Asian Nat. Prod. Res.* **2006**, *8*, 105–111.
(6) Grayer, R. J.; Veitch, N. C.; Kite, G. C.; Paton, A. J.; Garnock-Jones, P. *Phytochemistry* **2002**, *60*, 727–731.
(7) Wollenweber, E.; Mayer, K.; Roitman, J. N. *Phytochemistry* **1991**, *30*, 2445–2446.
(8) Nagao, T.; Abe, F.; Kinjo, J.; Okabe, H. *Biol. Pharm. Bull.* **2002**, *25*, 875–879.
(9) Horie, T.; Ohtsuru, Y.; Shibatat, K.; Yamashita, K.; Tsukayama, M.; Kawamukat, Y. *Phytochemistry* **1998**, *4I*, 865–874.
(10) Kinoshita, T.; Firman, K. *Phytochemistry* **1996**, *42*, 1207–1210.
(11) Ishii, T.; Yanagisawa, M. *Carb. Res.* **1998**, *313*, 189–192.
(12) Jimenez, C.; Riguera, R. *Nat. Prod. Rep.* **1994**, *11*, 591–606.
(13) Diaz, A. M. *Life Sci.* **2004**, *74*, 2515–2520.
(14) Setsuo, S.; Shgeya, S.; Norihiro, T.; Yiochi, N.; Keishi, N.; Madoka, I.; Isao, I. *Chem. Pharm. Bull.* **1990**, *38*, 411–414.
(15) Kinjo, J.; Uemura, H.; Nakamura, M.; Nohara, T. L. *Chem. Pharm. Bull.* **1994**, *42*, 1339–1341.
(16) Eudra/Q/92/021: Note for Guidance on Stability Testing of New Active Substances and Medicinal Products.
(17) Marzocco, S.; Piacente, S.; Pizza, C.; Oleszek, W.; Stochmal, A.; Pinto, A.; Sorrentino, R.; Autore, G. *Life Sci.* **2004**, *75*, 1491–501.
(18) Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. R. *Anal. Biochem.* **1982**, *126*, 131–138.

NP9002383