Polyphenols isolated from antiradical extracts of *Mallotus metcalfianus*

Céline Rivière [a,1], Van Nguyen Thi Hong [b,1], Luc Pieters [d], Bieke Dejaegher [c], Yvan Vander Heyden [c], Minh Chau Van [b], Joëlle Quetin-Leclercq [a,*]

[a] *Université Catholique de Louvain, Louvain Drug Research Institute, CHAM Unit, UCL 7230, Avenue E. Mounier 7, B-1200 Brussels, Belgium*
[b] *Institute of Natural Products Chemistry, Vietnamese Academy of Science and Technology, 18 Hoang Quoc Viet Road, Nghia Do Cau Giay, Hanoi, Viet Nam*
[c] *Vrije Universiteit Brussel, Department of Analytical Chemistry and Pharmaceutical Technology, Laarbeeklaan 103, B-1090 Brussels, Belgium*
[d] *University of Antwerp, Department of Pharmaceutical Sciences, Laboratory of Pharmacognosy and Pharmaceutical Analysis, Universiteitsplein, B-2610 Antwerp, Belgium*

ARTICLE INFO

ABSTRACT

Six flavonoids including two new flavones, luteolin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside), chry-soeriol-7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside) and a mixture of two pairs of diastereoisomeric flavonolignans, (±)-hydnocarpin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside)/(±)-hydnocarpin-D 7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside) with a 2:1 ratio were isolated from the whole plant of *Mallotus metcalfianus* Croizat, in addition to 10 known compounds. Their structures were evaluated on the basis of different spectroscopic methods, including extensive 1D and 2D NMR spectroscopy. Some extracts have moderate antimicrobial properties and interesting antiradical (DPPH) activity, as well as some compounds isolated from this species. Tannins were also identified in some active extracts.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

About 40 *Mallotus* species are localised in Vietnam and widely used in traditional medicine. Recently, a Vietnamese team studied *Mallotus apelta* and isolated seven new compounds belonging to benzopyrans which show very good NF-κB inhibition and cytotoxic activities against several human cell lines (Chau et al., 2005). During a systematic phytochemical examination of *Mallotus* species, we studied the chemical composition of *Mallotus metcalfianus* Croizat and evaluated some of its pharmacological properties. This species belongs to the Euphorbiaceae family and is a small tree growing in the evergreen forests of Vietnam and China, commonly known as "Ba bet do" in Vietnam, but we have no specific information about its traditional uses. This paper describes the isolation and characterization of six new flavonoids specifically named chry-soeriol-7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside) (**1**), luteolin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside) (**2**) and a mixture of two pairs of diastereoisomeric flavonolignans, (±)-hydnocarpin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside) ((±)-**3a**)/(±)-hydno-carpin-D 7-*O*-(4″-*O*-(*E*)-coumaroyl)-*β*-glucopyranoside) ((±)-**3b**) with a 2:1 ratio, as well as 10 known compounds. As the crude extract possessed antioxidant activities, these 16 natural products were tested for their antiradical activities specifically.

* Corresponding author. Tel.: +32 2 764 7254; fax: +32 2 764 7293.
E-mail address: joelle.leclercq@uclouvain.be (J. Quetin-Leclercq).
[1] Both authors contributed equally to this work.

2. Results and discussion

The crude methanolic extract of the whole *M. metcalfianus* plant was suspended in H_2O and extracted with *n*-hexane, chloroform and ethyl acetate. A combination of size exclusion chromatography, vacuum liquid and column chromatographies of the chloroform, ethyl acetate and aqueous phases led to the isolation of 12 pure compounds and a pure mixture of isomers. The structures of the known compounds were identified as quercetin 3-*O*-α-L-rhamnoside or quercitrin (**4**) (Fang et al., 2008), the major flavo-noid found in the ethyl acetate extract of this species, kaempferol 3-*O*-α-L-rhamnoside (**5**) (Markham and Chari, 1982), dihydroqu-ercetin 3-*O*-α-L-rhamnoside or astilbin (**6**) (Lu and Foo, 1999), quercetin 3-*O*-(2″-*O*-α-L-rhamnopyranosyl)-*β*-D-glucopyranoside or quercetin 3-*O*-*β*-neohesperidoside (**7**) (Zhou et al., 2005), kaempferol 3-*O*-(2″-*O*-α-L-rhamnopyranosyl)-*β*-D-glucopyranoside or kaempferol 3-*O*-*β*-neohesperidoside (**8**) (Carotenuto et al., 1996), but also *trans*-ferulic acid (**9**) (Ruan et al., 2007), methyl salicylate glucoside or methyl 2-*O*-*β*-D-glucopyranosylbenzoate (**10**) (Ushiyama and Furuya, 1989), a megastigmane, blumenol C glucoside (**11**) (Takeda et al., 1997), a phytosterol, friedelin-3α-ol or friedelinol (**12**) and a policosanol, 1-hexacosanol (**13**). This is the first report of these compounds in this species. Their structures were established using different physical methods including extensive 1D and 2D NMR spectroscopy, except for friedelinol and 1-hexacosanol identified by GC–MS and comparison with reference samples.

^{1}H NMR and ^{13}C NMR spectra of (**1**) and (**2**), displayed characteristic signals for a flavone nucleus, luteolin and chrysoeriol (the ^{1}H and ^{13}C NMR chemical shifts and coupling constants of **1** and **2** are listed in Table 1) substituted by a coumaric acid and a glucose.

Compound **1** was obtained as an amorphous yellow powder. The APCI–mass spectrometry (APCI–MS) gave an [M + H]$^+$ at m/z 595 with the positive-ion mode or an [M − H]$^-$ at m/z 593 with the negative ion mode indicating a mass 594 compatible with the molecular formula of $C_{30}H_{26}O_{13}$. Its molecular formula was confirmed by HRESIMS, which showed an ion at m/z [M − H]$^-$ 593.1248. The ^{1}H NMR spectrum of **1** exhibited an ABX system at δ_H 6.90 (d, J = 8.5 Hz, H-5′), 7.40 (d, J = 2.1 Hz, H-2′) and 7.42 (dd, J = 8.5, 2.1 Hz, H-6′) due to a 3′,4′-disubstitution pattern of the B ring. Two meta-coupled doublets at δ_H 6.83 (d, J = 2.0 Hz, H-8) and 6.52 (d, J = 2.0 Hz, H-6) were consistent with a 5,7-dioxygenated A ring. These resonances together with the singlet at δ_H 6.62 (H-3) and the corresponding carbon signals revealed the presence of the luteolin moiety as aglycone (Markham and Chari, 1982). Moreover, the ^{1}H NMR spectrum of **1** exhibited the characteristic signals belonging to an (E)-coumaroyl moiety (four aromatic protons characterized by two pairs of AB-type doublets and two trans-olefinic protons). Additionally, one anomeric proton resonance appeared at δ_H 5.15 (d, J = 7.7 Hz, H-1′ of β-glucose) indicative of the presence of one β-linked sugar unit. The ^{13}C NMR spectroscopic data confirmed the glycosidic unit in **1** exhibiting one anomeric carbon resonance at δ_C 100.0, which shows

correlation with the anomeric proton of glucose in the HSQC experiment. The results were supported by a GC–FID after methanolysis and silylation which confirmed the presence of a glucose unit. The significant deshielding of H-4″ (δ_H 4.97 dd, J = 9.3 Hz and 10 Hz) of glucose indicated that the coumaroyl unit was attached to C-4″ (OH) position of glucose. The chemical shift of the C-4 carbonyl at 182.6 ppm may be explained by a hydrogen-bond between the 5-OH and the C-4 carbonyl, leading to a downfield shift of this carbonyl (Markham and Chari, 1982). This indicates that the glucose unit is linked on the C-7(OH) position. Finally, HMBC experiments allowed the determination of all relevant interfragmental connectivities: significant cross-peaks were observed between the C-7 of the aglycone (δ_C 162.5) and the anomeric proton of the glucose, which confirmed that the glucose unit was attached to C-7(OH), and between the carbonyl of the coumaroyl unit (δ_C 167.5) and the H-4″ of the glucose, confirming the position of the coumaroyl substitution.

Thus, the structure of this new compound was established as luteolin 7-O-(4″-O-(E)-coumaroyl)-β-glucopyranoside) (Fig. 1). All 2D COSY, HSQC and HMBC experiments confirmed unambiguously this structure.

Compound **2** was obtained as an amorphous yellow powder. Its ^{13}C NMR spectrum displayed 31 resonances, which, in conjunction with the HRESIMS, indicated the formula $C_{31}H_{28}O_{13}$. The ^{1}H NMR spectrum of **2** exhibited again an ABX system at δ_H 6.93 (d, J = 8.5 Hz, H-5′), 7.50 (d, J = 2.1 Hz, H-2′) and 7.54 (dd, J = 8.5, 2.1 Hz, H-6′), two meta-coupled doublets at δ_H 6.86 (d, J = 2.0 Hz, H-8) and 6.52 (d, J = 2.0 Hz, H-6) and a singlet at δ_H 6.69 (H-3), but also a 3H singlet assignable to a methoxyl group at 3.96 (H-7′). A characteristic signal for carbonyl C-4 at δ_C 182.4 was detectable in the ^{13}C NMR. These resonances revealed the presence of the chrysoeriol moiety as aglycone. Moreover, the ^{1}H NMR spectrum of **2** exhibited again the characteristic signals belonging to an (E)-coumaroyl moiety (four aromatic protons characterized by two pairs of doublets and two trans-olefinic protons) and a β-linked glucose unit (one anomeric proton resonance at δ_H 5.15, d, J = 7.7 Hz, H-1″ of β-glucose) confirmed by the analysis by GC–FID of the product after methanolysis and silylation. The relevant interfragmental connectivities observed by HMBC were similar to compound **1**. Significant cross-peaks in the HMBC spectrum were observed between the C-7 of the aglycone (δ_C 162.8) and the anomeric proton of the glucose, which confirmed that the sugar unit was attached to C-7(OH), and between the carbonyl of the coumaroyl unit (δ_C 167.0) and the H-4″ of the glucose. Thus, the structure of this new compound was established as chrysoeriol 7-O-(4″-O-(E)-coumaroyl)-β-glucopyranoside) (Fig. 1). This was confirmed by 2D COSY, HSQC and HMBC connectivities.

These NMR data were also confirmed by comparison with the ^{1}H and ^{13}C NMR chemical shifts and coupling constants of apigenin 7-O-(4″-O-(Z)-coumaroyl)-β-glucopyranoside) (Zhao et al., 2007).

Compound **3** was obtained as an amorphous yellowish powder. The HRESIMS, m/z 771.1859 [M − H]$^-$, together with ^{13}C NMR spectroscopic data was used to deduce the molecular formula $C_{40}H_{36}O_{16}$. The ^{1}H NMR and ^{13}C NMR spectra of this compound displayed the typical signals of a 5–7-dihydroxy-substituted flavone (^{1}H and ^{13}C NMR chemical shifts and coupling constants of **3** are listed in Table 2) substituted by a coumaric acid and a glucose, similar to compound **1** except for protons of ring B of the flavone which were more deshielded. Furthermore, the APCI–MS^2 of the parent ion [M − H]$^-$ at m/z 771 gave a fragment ion at 593 corresponding to the mass of compound **1**. Compared to **1**, we observed three protons in the aromatic region which could be attributed to a second 1,3,4-trisubstituted aromatic ring, which may belong to a cinnamic alcohol group (Lee and Liu, 2003). The deshielded doublet δ_H 5.04 observed in the ^{1}H NMR spectrum suggested a benzylic methyne substituted by oxygen, and the trans-coupling

Table 1
^{1}H and ^{13}C NMR data for compounds **1** and **2** in CD$_3$OD.[a]

Position	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
Aglycone				
1				
2		165.4		165.4
3	6.62 s	102.8	6.69 s	103
4		182.6		182.4
5		161		162.4
6	6.52 d (2.0)	99.8	6.52 d (2.0)	99.8
7		162.5		162.8
8	6.83 d (2.0)	94.7	6.86 d (2.0)	94.6
9		157.4		157.9
10		105.9		105.4
1′		122.4		122.1
2′	7.4 d (2.1)	112.6	7.50 d (2.1)	109.4
3′		146		148.2
4′		148.2		150.7
5′	6.90 d (8.5)	115.3	6.93 d (8.5)	115.4
6′	7.42 dd (8.5/2.1)	119.1	7.54 dd (8.5/2.1)	120.4
7′			3.96 s	55.5
Glucose				
1″	5.15 d (7.7)	100.0	5.15 d (7.7)	100.1
2″	3.62 m	73.5	3.63 m	73.3
3″	3.76 m	74.2	3.77 m	73.6
4″	4.97 dd (10.0/9.3)	70.6	4.97 dd (10.0/9.3)	70.8
5″	3.80 m	75.0	3.81 m	75.5
6″	**6a** 3.59 m (12)	60.8	**6a** 3.60 m (12)	60.9
	6b 3.69 m (12)		**6b** 3.7 m (12)	
Coumaroyl moiety				
1*		125.8		125.6
2*	7.49 d (8.1)	129.7	7.48 d (8.1)	129.7
3*	6.82 d (8.1)	115.5	6.82 d (8.1)	115.2
4*		159.9		159.9
5*	6.82 d (8.1)	115.5	6.82 d (8.1)	115.2
6*	7.49 d (8.1)	129.7	7.48 d (8.1)	129.7
7* (β)	7.69 d (15.9)	145.9	7.69 d (15.9)	146.0
8* (α)	6.40 d (15.9)	113.2	6.40 d (15.9)	113.2
C=O		167.5		167.0

[a] 100 or 400 MHz, chemical shifts in ppm relative to TMS; ^{3}J in Hz. Assignments were established by DEPT, HSQC and HMBC data.
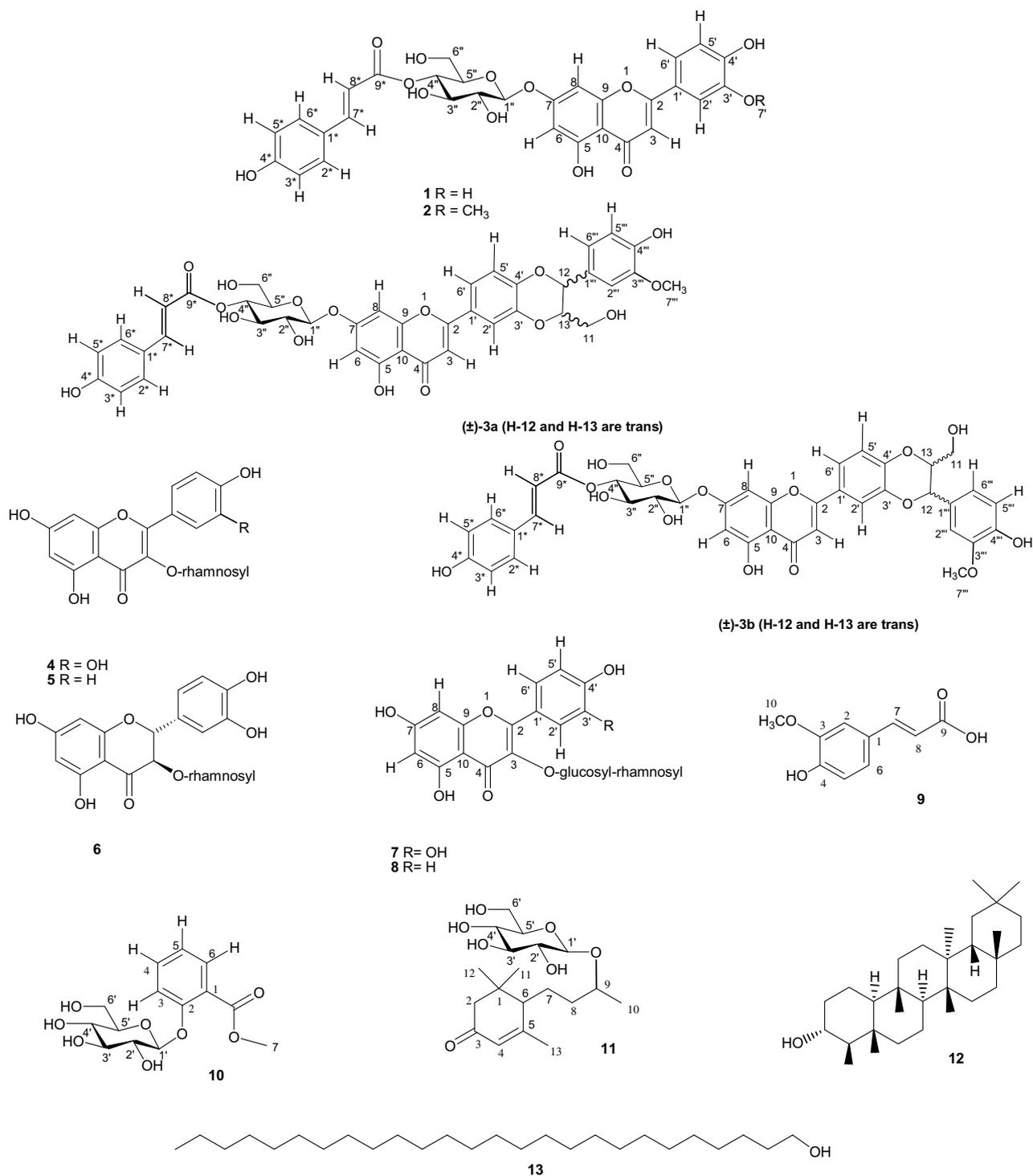
Fig. 1. Compounds isolated from *Mallotus metcalfianus* Croizat.

(J = 8.05 Hz) of the doublet indicated the presence of a trans-substituted 1,4-dioxane ring (Pettit et al., 2003). Thus, this compound is a flavonolignan. The COSY experiment revealed the coupling patterns of the oxygenated methyne and methene protons (H-11a,b, H-12 and H-13). These four protons could be assigned to a propanol moiety connected to a dioxane ring. The HMQC and HMBC (Table 2) analysis confirmed the position of 4″-*O*-(*E*)-coumaroyl-β-glucopyranoside at C-7, a methoxy group at C-3‴ and a phenol group at C-4‴ and confirmed that the 1,4-dioxane ring formed between the B ring of the flavone and the cin-

namic alcohol unit was situated at C-3′ and C-4′. In fact, the structure of this new compound corresponded to two pairs of diastereoisomeric flavonolignans, (±)-hydnocarpin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside) ((±)-**3a**)/(±)-hydnocarpin-D 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside) ((±)-**3b**) with a 2:1 ratio (Fig. 1). This ratio is easily determinable from the ratios of ^{1}H NMR doublets of H-12. In accordance with the literature (Guz and Stermitz, 2000), ^{1}H NMR doublets for H-12 in the derivative of hydnocarpin and in the derivative of hydnocarpin-D appeared at δ 4.97 and 5.04, respectively in DMSO-d_6. Even the HMBC correlations

Table 2
^{1}H and ^{13}C NMR data for compounds **3** in DMSO-d_6.[a]

Position	(±)-**3a**[b]		COSY	HMBC
	^{1}H	^{13}C		
Aglycone				
1				
2		163.5		
3	7.05 *s*	104.3		C-10/C-1'/C-2/C-4
4		182.1		
5		161.1		
6	6.56 *s*	99.6		C-8/C-10/C-5
7		162.9		
8	6.97 *s*	94.9		C-6/C-10/C-9/C-7
9		157.0		
10		105.5		
11	11a 3.45 *m*	60.1	H-13	
	11b 3.68 *m*			
12	5.04 *d* (8.05)[b]	76.4*	H-13	C-13/C-2'''/C-6'''/C-1'''
13	4.28 *m*[b]	78.0*	H-7'''/H-9'''a	
1'		123.6		
2'	7.78 *d* (2.0)	115.0		C-6'/C-4'/C-2
3'		143.7		
4'		147.2		
5'	7.18 *d* (8.5)	117.6	H-6'	C-1'/C-3'/C-4'
6'	7.71 *dd* (8.5/2.0)	120.1	H-5'	C-2'/C-4'
1'''		127.0		
2'''	7.11 *d* (2.5)	111.9		C-12/C-6'''/C-4'''
3'''		147.7		
4'''		147.3		
5'''	6.88 *d* (8.1)	115.4	H-6'''	C-1'''/C-3'''
6'''	6.94 *dd* (8.1/2.5)	120.7	H-5'''	C-2'''/C-4'''
7'''	3.85 *s*	55.8		C-3'''
Glucose				
1''	5.31 *d* (7.7)	99.6	H-2''	C-7
2''	3.47 *m*	73.2	H-1''	
3''	3.65 *m*	73.9	H-3''	
4''	4.85 *dd* (10.0/9.3)	70.8	H-3''/H-5''	
5''	3.88 *m*	74.8	H-4''	C-9*
6''	6a 3.41 *m* (12)	60.4		
	6b 3.54 *m* (12)			
Coumaroyl moiety				
1*		125.1		
2*	7.64 *d* (8.6)	130.4	H-3*	C-7*/C-6*/C-4*
3*	6.87 *d* (8.6)	115.8	H-2	C-5*/C-1*/C-4*
4*		159.9		
5*	6.87 *d* (8.6)	115.8	H-6*	C-3*/C-1*/C-4*
6*	7.64 *d* (8.6)	130.4	H-5*	C-7*/C-2*/C-4*
7* (β)	7.65 *d* (15.9)	145.1	H-8*	C-2*/C-6/C=O
8* (α)	6.47 *d* (15.9)	114.1	H-7*	C-1*
C=O		165.9		

[a] 100 or 400 MHz, chemical shifts in ppm relative to TMS; ^3J in Hz. Assignments were established by DEPT, HSQC and HMBC data.

[b] (±)-**3b** H-12 4.97 *d* (8.05)/C-12 75.9 – H-13 4.31 *m*/C-13 78.6.

spectrum did not give clear connectivities between H-12 or H-13 and C-4' and between H-12 or H-13 and C-3', ^{13}C NMR resonances for the hydnocarpin and the hydnocarpin-D derivatives are in accordance with the literature and confirm the presence of the mixture of the two isomers: for the hydnocarpin derivative, C-12 was at δ_C 76.4 and C-13 at δ_C 78.0 while for the hydnocarpin-D derivative, C-12 was at δ_C 75.9 and C-13 at δ_C 78.6 in DMSO-d_6 (Afifi et al., 1993; Guz and Stermitz, 2000; Stermitz et al., 2000).

2.1. Pharmacological activities

From our phytochemical results, *M. metcalfianus* is rich in flavonoids and phenolic compounds. These flavonoids were mainly present in the ethyl acetate extract while the aqueous fraction and the residue were richer in tannins (Table 3). Therefore, we decided to focus on the antimicrobial and antiradical properties of this plant. Tannins are known to possess general antimicrobial and antioxidant activities (Chung et al., 1998; Gu et al., 2008) and flavonoids are known for their antioxidant properties (Pietta,

Table 3
Tannin contents of *Mallotus metcalfianus* extracts (removed by a polyamide column).

Mallotus metcalfianus	Tannins	Percentage of removed tannins (on 21 mg extract) (%)
Ethyl acetate fraction	TG+; TC−	8.3
Aqueous fraction (MeOH 25%)	TG+; TC+	40
Aqueous fraction (MeOH 50%)	TG+; TC++	40.5
Residue	TG+; TC−	50

TG = gallic tannins, TC = catechic tannins, +: presence, −: absence.

2000). To determine the type of compounds responsible for the activity of these extracts, tannins were eliminated on a polyamide column according to the method described by Houghton and Raman (1998) (Table 3).

The antimicrobial activity of the *M. metcalfianus* fractions was evaluated on 20 strains. This activity is moderate. Fractions are not active on some Gram negative bacteria at the highest concentrations tested (1000 μg/ml for fractions with tannins and 500 μg/ml for fractions without tannins and the chloroformic subfraction

Table 4
Antimicrobial activities of crude methanolic extract and fractions of *Mallotus metcalfianus* (MIC and MAC in µg/ml).

		S. aur. 25923	S. aur. 104	E. fae. 29212	E. coli 25922	P. aer. 27853	M. mo. 180	M. mo. TP	Y. ent. 17098	Y. ent. 16998	C. alb.	C. trop.
MeOH extract	MIC	**1000**	>1000	>1000	>1000	**1000**	>1000	>1000	>1000	>1000	>1000	>1000
	MAC	>500	>1000	>1000	>1000	>500	>1000	>1000	>1000	>1000	**1000**	>1000
Hex. fraction	MIC	**1000**	**1000**	**1000**	**1000**	**1000**	>1000	>1000	>1000	>1000	**1000**	>1000
	MAC	>500	>500	>500	>500	>500	>1000	>1000	500	500	500	1000
CHCl₃ fraction	MIC	**1000**	>1000	>1000	**1000**	>1000	>1000	>1000	>1000	>1000	**1000**	>1000
	MAC	>500	>1000	**1000**	>500	>1000	>1000	>1000	>1000	**1000**	500	>1000
CHCl₃ subfraction D	MIC	**500**	>500	>500	>500	>500	>500	>500	>500	**500**	500	>500
	MAC	>200	>500	200	500	200	500	>500	>200	200	200	>500
AcOEt fraction	MIC	>1000	>1000	>1000	>1000	**1000**	>1000	>1000	>1000	>1000	**1000**	>1000
	MAC	>1000	**1000**	**1000**	**1000**	>500	>1000	**1000**	500	500	200	>1000
W50% fraction	MIC	**1000**	**1000**	>500	**1000**	**1000**	>1000	**1000**	>1000	>1000	**1000**	>1000
	MAC	>500	>500	**1000**	>1000	>500	**1000**	>500	200	200	500	>1000
W25% fraction	MIC	>1000	>1000	>1000	>1000	>1000	>1000	>1000	**500**	>1000	**500**	>1000
	MAC	>1000	>1000	>1000	>1000	>1000	>1000	>1000	**200**	**200**	< 500	>1000
Residue	MIC	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
	MAC	>1000	>1000	**1000**	>1000	>1000	**1000**	**1000**	**200**	**200**	1000	1000
Astilbin	MIC	>128	>128	**128**	>128	>128	>128	>128	>128	**128**	**128**	>128
	MAC	>128	>128	>128	>128	>128	>128	>128	**128**	>64	**64**	>128
Kaempferol	MIC	**128**	>128	>128	**128**	**128**	>128	>128	**128**	**128**	**128**	>128
3-O-α-L-Rhamnoside	MAC	>64	>128	**128**	>64	>64	**128**	**128**	>64	>64	**64**	>128
Quercitrin	MIC	>128	>128	>128	>128	**128**	>128	>128	**128**	**128**	**128**	**128**
	MAC	>128	>128	>128	>128	>64	**128**	**128**	>64	>64	**64**	>64
Tetracycline	MIC	**0.5**	>64	**64**	**0.5**	**32**	>64	**2**	**2**	**4**	>64	>64

Abbreviations: *S. aur.* 25923, *Staphylococcus aureus* ATCC 25923; *S. aur.* 104, *S. aureus* 104; *E. fae.* 29212, *Enterococcus faecalis* ATCC 29212; *E. coli* 25922, *Escherichia coli* ATCC 25922; *P. aer.* 27853, *Pseudomonas aeruginosa* ATCC 27853; *M. mo.* 180, *Morganella morganii* 180; *M. mo.* TP, *M. morganii* TP; *Y. ent.* 17098, *Yersinia enterocolitica* E 170/98; *Y. ent.* 16998, *Yersinia enterocolitica* E 169/98; *C. alb.*, *Candida albicans*; *C. trop.*, *Candida tropicalis*.

Table 5
Antiradical activities of *Mallotus metcalfianus* fractions.[a]

	Percentage of remaining DPPH (DPPH_REM)[a] 20 µg/ml
Tocopherol	**6.4% ± 5.1** (5)[*]
Mallotus metcalfianus	
MeOH extract	51.1% ± 14.5 (5)[*]
Hexane fraction	95.9% ± 9.4 (5)
Chloroform fraction	81.6% ± 7.8 (5)
Ethyl acetate fraction	**10.8% ± 6.5** (5)[*]
Ethyl acetate fraction without tannins	34.8% ± 14.4 (3)[*]
Aqueous fraction (MeOH 25%)	51.3% ± 12.5 (5)[*]
Aqueous fraction (MeOH 25%) without tannins	83.8% ± 7.8 (3)
Aqueous fraction (MeOH 50%)	**19.6% ± 12.3** (5)[*]
Aqueous fraction (MeOH 50%) without tannins	71.5% ± 2.8 (3)[*]
Residue	**47.6% ± 9.4** (5)[*]
Residue without tannins	90.3% ± 7.4 (3)

[a] Each value represents the mean ± SD of (*n*) determinations.
[*] Significantly different from the background control (methanol): $P < 0.05$.

D): *Escherichia coli* (clinical isolate), *Pseudomonas aeruginosa* ART 159, *Klebsiella pneumoniae* 116, *Klebsiella oxytoca* 118, *Enterobacter cloacae*, *E. aerogenes*, *Citrobacter freundii*, *Salmonella enteridis*, *Shigella sonnei*. Some fractions were effective on some strains at 1000 µg/ml and on at least 8 strains at 500 µg/ml, some MAC (MAC, minimal active concentration) were as low as 200 µg/ml (Table 4). This activity in most cases (polar extracts) may be explained at least partly by the presence of tannins as minimal inhibitory concentration (MIC) increases after their removal. Hexanic and chloroformic fractions, particularly subfraction D (MAC = 200 µg/ml on 4 strains among which *P. aeruginosa* ATCC 27853 and MIC = 500 µg/ml on 3 strains), show also an interesting activity. Pure isolated major flavonoids (astilbin, kaempferol 3-*O*-α-L-rhamnoside and quercitrin) have a moderate activity (MIC = 128 µg/ml on some strains).

Concerning the antiradical activity, for the ethyl acetate fraction which was the most active, we observed that tannins were only responsible for a small part of the activity which seems to be mainly due to flavonoids. In fact, the elimination of tannins in this fraction only slightly decreased the antiradical properties. On the contrary, tannins seem to be responsible for a large part of the antioxidant activities of the residue and the aqueous fraction: their elimination greatly decreased the activity (Table 5). The antioxidant activity of flavonoids is due to their ability (1) to reduce reactive oxygen species formation either by inhibition of enzymes or chelating trace elements involved in free radical production; (2) to scavenge free radicals; and (3) to upregulate or protect antioxidant defenses. The major determinants for radical-scavenging capability are the presence of a catechol group in ring B and a 2,3-double bond conjugated with the 4-oxo-group. Flavonols are more potent than the corresponding flavones because of the presence of the 3-hydroxyl group (Pietta, 2000). If important structure-activity relationships of the antioxidant activity of flavonoids have been established, little information is available concerning pharmacological activities of flavonoids substituted by a derivative of cinnamic acid. So, we tested the different pure compounds isolated from *M. metcalfianus* and different reference samples (flavonoids and cinnamic acid derivatives) for their antiradical activities in order to discuss about structure-activity relationships of these products (Table 6). We observed that quercetin 3-*O*-β-neohesperidoside shows about 50% reduction of activity compared to rutin. This decrease of activity can be due to the different position of rhamnose on glucose. Kaempferol 3-*O*-β-neohesperidoside, having an OH less on the B ring, shows a very moderate activity, in agreement with the literature (Gamez et al., 1998) and structure-activity relationships mentioned above. The new flavonolignans **3** were not very active. This lack of activity could be explained by the cyclization of the catechol group of the B ring of the flavone. Indeed, by comparison with luteolin, luteolin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside) was found to be moderately active in the DPPH assay. The substitution of the flavone by a coumaric acid could explain this decrease of activity, as coumaric acid does not show a real antioxidant activity unlike caffeic acid. Chrysoeriol 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside) is less active than luteolin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside), probably because of the loss of phenol function in position 3′, replaced by a methoxy group. Methyl 2-*O*-β-D-glucopyranosylbenzoate, blumenol C

Table 6
Antiradical activities of *Mallotus metcalfianus* compounds and reference samples.[a]

	IC$_{50}$ (µg/ml)[a]	MM (g/mol)	IC$_{50}$ (µM)
Isolated compounds			
Luteolin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside) (**1**)	10.0 ± 1.6 (3)	594	16.8
Chrysoeriol 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside) (**2**)	52.2 ± 0.2 (3)	608	85.8
Mixture of (±)Hydnocarpin/Hydnocarpin-D 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside) (±**3a,b**)	>40 (3)	772	>51.8
Quercitrin (**4**)	2.3 ± 1.4 (3)	448	5.1
Kaempferol 3-*O*-α-ʟ-rhamnoside (**5**)	3.6 ± 0.2 (3)	432	8.3
Astilbin (**6**)	10.3 ± 1.2 (3)	450	22.9
Quercetin 3-*O*-β-neohesperidoside (**7**)	4.3 ± 1.5 (3)	610	7.1
Kaempferol 3-*O*-β-neohesperidoside (**8**)	67,2 ± 1.7 (3)	594	113.1
trans-Ferulic acid (**9**)	11.5 ± 0.9 (3)	194	59.3
Methyl 2-*O*-β-ᴅ-glucopyranosylbenzoate (**10**)	>40 (3)	314	>127.4
Blumenol C glucoside (**11**)	>40 (3)	432	>92.6
Friedelinol (**12**)	>40 (3)	428	>93.4
1-Hexacosanol (**13**)	>40 (3)	382	>104.7
Reference samples			
Tocopherol	4.9 ± 1.0 (6)	402	12.2
Rutin	2.5 ± 1.0 (3)	610	4.1
Quercetin	2.2 ± 0.9 (3)	302	7.3
Kaempferol	2.5 ± 0.7 (3)	286	8.7
Chrysoeriol	>40 (3)	286	>139.9
Luteolin	1.8 ± 0.2 (3)	300	6.3
Ferulic acid	3.7 ± 1.2 (3)	194	19.1
Coumaric acid	>40 (3)	164	>243.9
Caffeic acid	3.2 ± 1.4 (3)	180	17.8
Friedelin	>40 (3)	426	>93.9

[a] Each value represents the mean ± SD of (*n*) determinations.

glucoside, friedelinol and 1-hexacosanol were found to have only a low activity. Friedelin (hydroxyl function of friedelinol in position 3 replaced by a ketone) was not more active than friedelinol.

3. Conclusion

Although the Vietnamese *Mallotus* species have been reported to contain triterpenoids, benzopyrans, flavonoids, coumarinolignoids, dimeric chalcones and phloroglucinol derivatives (Kiem et al., 2004, 2005; Chau et al., 2004; Cheng and Chen, 2000; Tanaka et al., 1998; Supudompol et al., 2004), this is the first report of the presence of flavonolignans in this genus. Flavonolignans do not appear to have been reported from species in the Euphorbiaceae previously. Because of the importance of tannins and flavonoids in this species, we focused on the antiradical activity. The new flavonolignans (**3**) did not show interesting activity but some isolated flavonoids possess high effects and confirm previous structure-activity relationships. The presence of a substitution by coumaric acid also decreased the activity.

4. Experimental

4.1. General

UV spectra were recorded on Uvikon 933 UV/Vis spectrophotometer. IR spectra (KBr) were measured on a Perkin–Elmer FTIR 286 spectrometer. NMR spectra were recorded on a Bruker Avance DRX-400 spectrometer in CDCl$_3$, CD$_3$OD or DMSO at 400 MHz (^{1}H) and 100 MHz (^{13}C), at 30 °C. A combination of COSY, HMQC, HMBC and ROESY experiments was used when necessary for the assignment of ^{1}H and ^{13}C chemical shifts. The ^{1}H and ^{13}C chemical shift values are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as internal standard, and the coupling constants are in Hertz. Mass spectra in positive or negative-ion mode were acquired with a Thermofinnigan LCQ Advantage ion trap mass spectrometer, equipped with an APCI or ESI source. High-resolution ESIMS were measured on a Thermo Scientific LTQ orbitrap XL mass spectrometer. Data acquisition and processing were performed with Xcalibur software.

The gas chromatograph was a TRACE GC 2000 series (ThermoQuest, Italy), equipped with an autosampler AS2000 (ThermoQuest). The GC system was interfaced to a Trace MS mass spectrometer (ThermoQuest) operating in the electron-impact mode. Chromatographic separations (friedelinol and 1-hexacosanol) were performed on a capillary nonpolar column (DX-XLB; column length 15 m × 0.25 mm with a 0.25 µm film thickness) from J&W Scientific (Agilent Technologies, USA, Serial No. US4871423B). Helium was used as carrier gas at a flow rate of 1.2 ml/min. Samples (1 or 5 µl, stock solutions at 1 mg in 1.5 ml isopropanol) were injected without split mode. The injector temperature was set at 300 °C and the oven programmed from 50 °C to 320 °C (10 °C/min) and this last temperature was maintained for 10 min. The electron energy was 70 eV and the ion source was at 250 °C. Samples were analysed in a full-scan mode (36–739 amu).

Analytical TLC was performed on precoated silica gel 60 F$_{254}$ plates (Merck) and detection was achieved by spraying with different reagents (sulfuric acid 10% or sulfuric anisaldehyde or phosphomolybdic acid or fast blue salt B or NP/PEG or Dragendorff), followed by heating 5 min at 105 °C depending on the used reagent. Vacuum liquid chromatography (VLC) was performed on silica gel 60 (Merck, >63 µm). Column chromatographies were performed on silica gel normal phase 60 H (Merck, 40–63 µm, 230–400 mesh), on silica gel reversed phase (YMC RP-18 resins, 30–50 µm, Fujisilisa Chemical Ltd.), on polyamid (MN polyamid SC 6, particle size: 0.05–0.16 mm), on Toyopearl HW-40S (Tosoh) and/or on dianion. Normal phase preparative TLC was performed on precoated silica gel 60 F$_{254}$ glass plates measuring 20 × 20 cm. In case of preparative TLC, the chromatograms were air dried after development and bands corresponding to compounds were scraped off based on their absorbance of UV light (254 nm and 366 nm) and R_f values obtained from the analytical TLC. The scrapings were then washed repeatedly with methanol and CH$_2$Cl$_2$, filtered and concentrated.

4.2. Plant material

The whole *M. metcalfianus* plant was collected in May 2006 in the HaGiang mountains in Vietnam and identified by Dr. Nguyen Nghia Thin, Institute of Ecology and Biological Resources, VAST, Vietnam. An authentic sample was deposited at the Institute of Natural Products Chemistry, VAST, Vietnam (Voucher Number: NT03). The plant material was dried at room temperature and powdered.

4.3. Extraction and isolation

The dried and powdered whole plant of *M. metcalfianus* (500 g) was extracted three times with MeOH (2 l) repeatedly by maceration to give 50 g extract, which was suspended in water (2 l) and extracted sequentially at room temperature with increasing polarity solvents (2 l) giving *n*-hexane (9 g), chloroform (6 g) and ethyl acetate (5 g) extracts after removal of solvents. Isolation of pure compounds was achieved by different chromatographie procedures.

The chloroformic fraction (5 g) was subjected to VLC on silica gel normal phase using a gradient of CH$_2$Cl$_2$/EtOAc (90/10–0/100) followed by increasing concentrations of MeOH in EtOAc to give 10 fractions (A–J). Fractions B and C were assembled (415 mg) and rechromatographed on silica gel normal phase using CH$_2$Cl$_2$/MeOH (50/1) as eluant to yield 8 fractions. Fraction 3 gave friedelinol (**12**) and fraction 5 1-hexacosanol (**13**). Fractions D and E were assembled (200 mg) and rechromatographied on silica gel normal phase using CH$_2$Cl$_2$/MeOH (50/1) as eluant to yield 13 fractions. Fraction 4 also gave friedelinol (**12**) and fraction 6 1-hexacosanol (**13**). Fractions 10 to 13 were assembled and were submitted to column chromatography on silica gel normal phase using CH$_2$Cl$_2$/MeOH (6/1) followed by preparative TLC on silica gel normal phase using CH$_2$Cl$_2$/MeOH (6/1) in order to obtain (*E*)-ferulic acid (**9**).

The ethyl acetate fraction (5 g) was chromatographied on silica gel normal phase using CHCl$_3$/MeOH (7/1). Nine fractions were obtained. Fractions 2 and 3 were assembled (500 mg) and rechromatographied on silica gel normal phase using CHCl$_3$/acetone (5/1) as eluant to yield 10 fractions (A–J). Fractions G to I were assembled and rechromatographied on RP18 phase with MeOH/H$_2$O (1/1) in order to obtain chrysoeriol 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside (**2**) (3 mg) and the mixture of (±)-hydnocarpin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside ((±)-**3a**) and (±)-hydnocarpin-D 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside ((±)-3 b) (7 mg). Fraction J was chromatographied on RP18 phase with MeOH/acetone/H$_2$O (1/1/1.5) in order to obtain luteolin 7-*O*-(4″-*O*-(*E*)-coumaroyl)-β-glucopyranoside (**1**) (4 mg) and kaempferol 3-*O*-α-ʟ-rhamnoside (**5**). Fractions 4 and 5 coming from the first chromatography separation were assembled (1.4 g) and rechromatographied on RP18 using MeOH//H$_2$O (1/1.5) as solvent system. Two compounds were obtained: quercitrin (**4**) and astilbin (**6**).

The aqueous phase was subject to chromatography on dianion using MeOH/H$_2$O (25% MeOH (1 l), 50% MeOH (1 l) and 75% MeOH (1 l)) as eluent yielding three fractions: A$_{aq}$ (0.4 g), B$_{aq}$ (2 g) and C$_{aq}$ (1.5 g). Quercetin 3-*O*-β-neohesperidoside (**7**) (17 mg) was obtained from B$_{aq}$ by column chromatography on polyamid using a gradient of H$_2$O/MeOH (100/0–0/100). C$_{aq}$ was further fractionated by silica gel column chromatography using a gradient of CHCl$_3$/MeOH (10/1 to 1/1) to give four major subfractions (C1–C4). Subfraction C2 (330 mg) was purified by column chromatography on RP-18 with acetone/H$_2$O (1/1) as mobile phase in order to afford four fractions. The first fraction (45 mg) was purified on silica gel normal phase using CH$_2$Cl$_2$/MeOH (8/1) in order to obtain two products: methyl 2-*O*-β-ᴅ-glucopyranosylbenzoate (**10**) (1.5 mg) and blumenol C glucoside (**11**) (7 mg). This last molecule was also obtained from the second fraction (30 mg) by chromatography on silica gel normal phase using CH$_2$Cl$_2$/MeOH (6/1). Subfraction C4

(110 mg) was purified by column chromatography on silica gel normal phase using CHCl$_3$/MeOH/H$_2$O (4/1/0.1). Quercitrin (**4**) and kaempferol 3-*O*-β-neohesperidoside (**8**) were obtained.

4.3.1. Luteolin 7-O-(4″-O-(E)-coumaroyl)-β-glucopyranoside (**1**)

Yellow amorphous powder; UV λ_{max} (MeOH) nm (log ε): 315 (3.55), 268 (3.42), 216 (3.58); IR (KBr) ν_{max} 3430 (OH), 1698 (conjugated ester C=O), 1650 (C=O flavone), 1631 (C=C), 1607, 1515 and 1496 (aromatic rings), 1077 (C–O–C) cm^{-1}; for ^{13}C and ^{1}H NMR spectra, see Table 1; APCI–MS (negative-ion mode) *m/z*: 593 [M − H]$^-$; APCI–MS (positive-ion mode) *m/z*: 595 [M + H]$^+$; HR–ESI–Orbitrap–MS (negative-ion mode) *m/z*: 593.1248 [M − H]$^-$ (calcd. 593.1296 for C$_{30}$H$_{25}$O$_{13}$ [M − H]$^-$).

4.3.2. Chrysoeriol 7-O-(4″-O-(E)-coumaroyl)-β-glucopyranoside (**2**)

Yellow amorphous powder; UV λ_{max} (MeOH) nm (log ε): 317 (4.35), 269 (4.19), 212 (4.44); IR (KBr) ν_{max} 3420 (OH), 1696 (conjugated ester C=O), 1653 (C=O flavone), 1638 (C=C), 1617, 1515 and 1497 (aromatic rings), 1077 (C–O–C), 1028 (O–CH$_3$) cm^{-1}; for ^{13}C and ^{1}H NMR spectra, see Table 1; APCI–MS (negative ion mode) *m/z*: 607 [M − H]$^-$; APCI–MS (positive-ion mode) *m/z*: 609 [M + H]$^+$; HR–ESI–Orbitrap–MS (negative-ion mode) 607.1423 (calcd. 607.1452 for C$_{31}$H$_{27}$O$_{13}$ [M − H]$^-$).

4.3.3. Mixture of (±)-hydnocarpin 7-O-(4″-O-(E)-coumaroyl)-β-glucopyranoside ((±)-3a)/(±)-hydnocarpin-D 7-O-(4″-O-(E)-coumaroyl)-β-glucopyranoside ((±)-**3b**)

Yellowish amorphous powder; UV λ_{max} (MeOH) nm (log ε): 320 (4.45), 273 (4.27), 211 (4.58); IR (KBr) ν_{max} 3452 (OH), 1696 (conjugated ester C=O), 1653 (C=O flavonolignan), 1635 (C=C), 1615, 1515 and 1497 (aromatic rings), 1077 (C–O–C), 1028 (O–CH$_3$) cm^{-1}; for ^{13}C and ^{1}H NMR spectra, see Table 2; APCI–MS (negative ion mode) *m/z*: 771 [M − H]$^-$; APCI–MS (positive-ion mode) *m/z*: 773 [M + H]$^+$; HR–ESI–Orbitrap–MS 771.1859 (calcd. 771.1926 for C$_{40}$H$_{35}$O$_{16}$ [M − H]$^-$).

4.4. Sugar analysis

The monosaccharide composition analysis was performed after acid-catalysed methanolysis and analysis of the liberated *O*-methyl-glycosides as trimethylsilylated derivatives. Samples (0.3 mg) were dried and 0.15 ml of the methanolysis reagent (MeOH–HCl, 0.5 N, kit, Supelco) was added. The sample solution was kept at 80 °C for 17 h. After methanolysis, samples were evaporated to dryness under a light stream of nitrogen, followed by the addition of 0.15 ml of silylation reagent (HMDS/TMCS/pyridine: 3/1/9, kit, Supelco). The silylation was left to complete at 60 °C for 30 min before analysis. Samples (1 μl, stock solution at 0.3 mg in 100 μl anhydrous acetonitrile) were injected with split mode (split flow 41 ml/min, ratio 27). The volatile derivatives were separated by gas chromatography on a Focus GC Interscience Thermofinigan chromatograph equipped with a 30 m × 0.53 mm CP-Sil5 CB Low bleed/MS capillary column, 1.5 μm film phase (Chrompack). Helium was used as carrier gas at a flow rate of 1.5 ml/min. The injector temperature was set at 250 °C and the oven programmed from 130 °C (3 min) to 300 °C (3 °C/min) and this last temperature was maintained for 30 min. Samples were analysed with a FID detector.

4.5. Elimination of tannins

The presence of tannins in the ethyl acetate, aqueous fractions and in the residue was revealed by a colorimetric test with 1% FeCl$_3$. These fractions (21 mg) were dissolved in water and applied to a 5 g polyamide column in order to eliminate the tannins. Elution was performed with water (10 ml), water/MeOH (10 ml), and MeOH (40 ml) until the eluate was clear. The eluate was then

evaporated under reduced pressure. Fractions without tannins were tested.

4.6. Antimicrobial activity

The test was performed as described by Gbaguidi et al. (2005). Before testing, the extracts, fractions or compounds were solubilised in MeOH to give 4 mg/ml, 10 mg/ml and 20 mg/ml stock solutions for extracts and fractions, and 1.28 mg/ml and 2.56 mg/ml for pure compounds. Tetracycline (Sigma, Belgium), used as positive control, was solubilised in water to give 1.28 mg/ml stock solution. The medium was the Muller–Hinton 2 agar (bioMerieux, France) prepared at 38 g/l, autoclaved and maintained at 45 °C. The agar dilution method was used to determine the minimal inhibitory concentration (MIC – the minimum concentration completely inhibiting growth of microorganism) and the minimal active concentration (MAC – the minimal concentration reducing the growth of the microorganism as compared to controls) for extracts, fractions and pure compounds. The microorganisms were grown overnight on tryptone soya broth (Oxoid Ltd., England). Inocula of 10^4 colony forming units (CFU) were spotted with a multipoint inoculator A400 (Denley Instruments Ltd., England) on 19 ml Muller–Hinton 2 agar supplemented with the extracts or antibiotics (1 ml) giving concentrations ranging from 200 to 1000 μg/ml for the extracts, from 0.5 to 64 μg/ml for tetracycline, and at 64 and 128 μg/ml for pure compounds. The plates were incubated for 24 h at 37 °C. Tests were performed at least in duplicate. A blank (MeOH used to dissolve extracts and compounds) was included.

Antimicrobial activity was tested on the following reference strains from international collections:

– Gram positive bacteria: *Staphylococcus aureus* ATCC 25923, *S. aureus* 104, *Enterococcus faecalis* ATCC 29212.
– Gram negative bacteria: *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

In addition, we also used strains from our laboratory collection obtained by clinical isolation.

– Gram negative bacteria: *E. coli*, *P. aeruginosa* ART 159, *K. pneumoniae* 116, *K. oxytoca* 118, *E. cloacae*, *E. aerogenes*, *Citrobacter freundii*, *Morganella morganii* 180, *M. morganii* TP, *S. enteridis*, *S. sonnei*, *Yersinia enterocolitica* E 170/98, *Y. enterocolitica* E 169/98.
– Saccharomycetes fungi: *Candida albicans*, *Candida tropicalis*.

4.7. Antioxidant activity: DPPH Assay

The test was performed as described by Aquino et al. (2001). The antiradical activities of extracts, fractions, pure compounds and tocopherol (positive control, Sigma, Belgium) were determined using the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH°, Sigma, Belgium).

In its radical form, DPPH° has an absorption band at 515 nm, which disappears upon reduction by an antiradical compound. An aliquot (50 μl) of the MeOH solution containing extract or positive control was added to 2.5 ml of daily prepared DPPH° solution (25 μg/ml in MeOH). An equal volume (50 μl) of the vehicle alone (MeOH) was added to control tubes (DPPH°$_0$). Absorbances at 515 nm were measured on a Uvikon 933 spectrophotometer 20 min after starting the reaction. Extracts were tested at a concentration of 20 μg/ml. Pure compounds and reference samples were tested from 40 μg/ml to 0.4 μg/ml, in order to determinate IC$_{50}$.

The DPPH° concentration in the reaction medium was calculated from a calibration curve obtained after linear regression of the absorbance at 515 nm, at concentrations ranging from 1 to 50 μg/ml of DPPH°.

The percentage of remaining DPPH° (DPPH°$_{REM}$) was calculated as follows:

$$\%DPPH°_{REM} = [DPPH°_{20\ min}]/[DPPH_0] \times 100$$

All experiments were carried out in triplicate.

4.8. Statistical analysis

Statistical calculations were carried out with GraphPad Prism 4. Results are expressed as the mean ± SD (Standard Deviation) of (n) independent experiments with individual values. The unpaired Student's t-test was used for the comparison of means; P values <0.05 were considered as significantly different from the control.

References

Afifi, M.S.A., Ahmed, M.M., Pezzuto, J.M., Kinghorn, A.D., 1993. Cytotoxic flavonolignans and flavones from *Verbascum sinaiticum* leaves. Phytochemistry 34, 839–841.

Aquino, R., Morelli, S., Rosaria Lauro, M., Abdo, S., Saija, A., Tomaino, A., 2001. Phenolic constituents and antioxidant activity of an extract of *Anthurium versicolor* leaves. J. Nat. Prod. 64, 1019–1023.

Carotenuto, A., De Feo, V., Fattorusso, E., Lanzotti, V., Magno, S., Cicala, C., 1996. The flavonoids of *Allium ursinum*. Phytochemistry 41, 531–536.

Chau, V.M., Phan, V.K., Nguyen, H.N., Hoang, T.H., Lee, J.J., Kim, Y.H., 2004. Chemical investigations and biological studies of *Mallotus apelta*. V. Flavonoids and other compounds from *Mallotus apelta*. Tap Chi Hoa Hoc 42, ii–iii.

Chau, V.M., Phan, V.K., Nguyen, H.N., Hoang, T.H., Lee, J.J., Kim, Y.H., 2005. Chemical investigations and biological studies of *Mallotus apelta*. IV-constituents with inhibitory activity against NFAT transcription and NF-κB activation from *Mallotus apelta*. Tap Chi Hoa Hoc 43, 773–777.

Cheng, X.F., Chen, Z.L., 2000. Coumarinolignoids of *Mallotus apelta*. Fitoterapia 71, 341–342.

Chung, K.T., Wong, T.Y., Wei, C.I.Y., Huang, Y.W.Y., Lin, Y.Y., 1998. Tannins and human health: a review. Crit. Rev. Food Sci. Nutr. 38, 421–464.

Fang, S.H., Rao, Y.K., Tzeng, Y.M., 2008. Anti-oxidant and inflammatory mediator's growth inhibitory effects of compounds isolated from *Phyllanthus urinaria*. J. Ethnopharmacol. 116, 333–340.

Gamez, E.J.C., Luyengi, L., Lee, S.K., Zhu, L.F., Zhou, B.N., Fong, H.H.S., Pezzuto, J.M., Kinghorn, A.D., 1998. Antioxidant flavonoid glycosides from *Daphniphyllum calycinum*. J. Nat. Prod. 61, 706–708.

Gbaguidi, F., Accrombessi, G., Moudachirou, M., Quetin-Leclercq, J., 2005. HPLC quantification of two isomeric triterpenic acids isolated from *Mitracarpus scaber* and antimicrobial activity on *Dermatophilus congolensis*. J. Pharm. Biomed. Anal. 39, 990–995.

Gu, H.F., Li, C.M., Xu, Y.J., Hu, W.F., Chen, M.H., Wan, Q.H., 2008. Structural features and antioxidant activity of tannin from persimmon pulp. Food Res. Int. 41, 208–217.

Guz, N.R., Stermitz, F.R., 2000. Synthesis and structures of regioisomeric hydnocarpin-type flavonolignans. J. Nat. Prod. 63, 1140–1145.

Houghton, P.J., Raman, A., 1998. Laboratory Handbook for the Fractionation of Natural Extracts. Chapman and Hall, London.

Kiem, P.V., Chau, V.M., Huong, H.T., Nam, N.H., Lee, J.J., Kim, Y.H., 2004. Pentacyclic triterpenoids from *Mallotus apelta*. Arch. Pharm. Res. 27, 1109–1113.

Kiem, P.V., Dang, N.H., Bao, H.V., Huong, H.T., Chau, V.M., Huong, L.M., Lee, J.J., Kim, Y.H., 2005. New cytotoxic benzopyrans from the leaves of *Mallotus apelta*. Arch. Pharm. Res. 28, 1131–1134.

Lee, D.Y.W., Liu, Y., 2003. Molecular structure and stereochemistry of sylibin A, silybin B, isosilybin A, isosilybin B, isolated from *Sylibum marianum* (Milk Thistle). J. Nat. Prod. 66, 1171–1174.

Lu, Y., Foo, L.Y., 1999. The polyphenol constituents of grape pomace. Food Chem. 65, 1–8.

Markham, K.R., Chari, V.M., 1982. ^{13}C NMR spectroscopy of flavonoids. In: Harborne, J.B., Mabry, T.J. (Eds.), The Flavonoids: Advances in Research. Chapman and Hall, London, pp. 19–134.

Pietta, P.G., 2000. Flavonoids as antioxidants. J. Nat. Prod. 63, 1035–1042.

Pettit, G.R., Meng, Y., Stevenson, C.A., Doubek, D.L., Knight, J.C., Cichacz, Z., Pettit, R.K., Chapuis, J.C., Schmidt, J.M., 2003. Isolation and structure of palstatin from the amazon tree *Hymeneae palustris*. J. Nat. Prod. 66, 259–262.

Ruan, H.L., Zhou, X.F., Zhang, Y.H., Pi, H.F., Wu, J.Z., Sun, H.D., 2007. Ferulic acid esters from *Euphorbia hylonoma*. Fitoterapia 78, 72–73.

Stermitz, F.R., Tawara-Matsuda, J., Lorenz, P., Mueller, P., Zenewicz, L., Lewis, K., 2000. 5′-Methoxyhydnocarpin-D and pheophorbide A: *Berberis* species components that potentiate berberine growth inhibition of resistant *Staphylococcus aureus*. J. Nat. Prod. 63, 1146–1149.

Supudompol, B., Likhitwitayawuid, K., Houghton, P.J., 2004. Phloroglucinol derivatives from *Mallotus pallidus*. Phytochemistry 65, 2589–2594.

Takeda, Y., Zhang, H., Masuda, T., Honda, G., Otsuka, H., Sezik, E., Yesilada, E., Sun, H., 1997. Megastigmane glucosides from *Stachys byzantina*. Phytochemistry 44, 1335–1337.

Tanaka, T., Ito, T., Linuma, M., Takahashi, Y., Naganawa, H., 1998. Dimeric chalcone derivatives from *Mallotus philippinensis*. Phytochemistry 48, 1423–1427.

Ushiyama, M., Furuya, T., 1989. Studies on plant tissue culture. Part 63. Glycosylation of phenolic compounds by root culture of *Panax ginseng*. Phytochemistry 28, 3009–3013.

Zhao, J., Pawar, R.S., Ali, Z., Khan, I.A., 2007. Phytochemical investigation of *Turnera diffusa*. J. Nat. Prod. 70, 289–292.

Zhou, X., Peng, J., Fan, G., Wu, Y., 2005. Isolation and purification of flavonoid glycosides from *Trollius ledebouri* using high-speed counter-current chromatography by stepwise increasing the flow-rate of the mobile phase. J. Chromatogr. A 1092, 216–221.