Planta Medica

Journal of Medicinal Plant and Natural Product Research

Publishers

Georg Thieme Verlag KG
Stuttgart · New York
Rüdigerstraße 14
D-70469 Stuttgart
Postfach 30 11 20
D-70451 Stuttgart

Thieme Publishers
333 Seventh Avenue
New York, NY 10001, USA
www.thieme.com

Lignans and Other Constituents from the Roots of the Vietnamese Medicinal Plant *Pseuderanthemum palatiferum*

Huong Doan Thi Mai[1], **Hang Nguyen Thi Minh**[1], **Van Cuong Pham**[1], **Kim Nga Bui**[2], **Van Hung Nguyen**[1], **Van Minh Chau**[1]

[1] Institute of Marine Biochemistry – VAST, Hanoi, Vietnam

[2] 7-Nga Tay Ninh Company, Tay Ninh, Vietnam

Abstract

Two new lignans, palatiferin A (**1**) and palatiferin B (**2**), were isolated from the roots of *Pseuderanthemum palatiferum*, together with five known triterpenes, epifriedelanol (**3**), lupeol (**4**), lupenone (**5**), betulin (**6**), pomolic acid (**7**), and a dipeptide asperglaucide (**8**). Their structures were established from 2D NMR and mass spectroscopy. The absolute configuration of **1** and **2** was proposed based on the comparison of their optical rotation activities with those of compounds with similar structures such as wodeshiol and paulownin. The new lignans, palatiferin A (**1**) and palatiferin B (**2**) exhibited a moderate cytotoxicity against KB and HepG2 cell lines. However, betulin and lupeol, two abundant compounds from the roots of *P. palatiferum*, showed cytotoxic and antimicrobial activities.

Key words

Acanthaceae · *Pseuderanthemum palatiferum* · palatiferin · betulin · lupeol · cytotoxicity · antimicrobial

Abbreviations

MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

FBS: fetal bovine serum

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Pseuderanthemum palatiferum (Nees) Radlk. is a medicinal plant and belongs to the Acanthaceae family. In Vietnam, *P. palatiferum* is known under the names Hoan-Ngoc or Xuan-Hoa. This plant has been used in Vietnamese traditional medicine for treatment of many diseases including hypertension, diarrhea, arthritis, nephritis, and diabetes [1,2]. The whole plant of *P. palatiferum* is commercialized under the name "Hoan-Ngoc tea" for health promotion and cancer prevention. Since it is useful in traditional medicine, this plant has attracted the attention of many scientists, and several triterpenoids and flavonoids have been reported from the leaves of *P. palatiferum* [3,4]. In our program of research on Vietnamese medicinal plants, we report here the isolation and structural elucidation of the two new lignans, palatiferin A (**1**) and palatiferin B (**2**) from the roots of *P. palatiferum*, along with six known compounds, epi-friedelanol (**3**) [5], lupeol (**4**) [6], betulin (**5**) [7], lupenone (**6**) [8], pomolic acid (**7**) [9], and asperglaucide (**8**) [10] (**Fig. 1**). Biological screening indicated that the two new compounds, palatiferin A (**1**) and palatiferin B (**2**), had a moderate cytotoxicity against KB and HepG2 cells. Whereas betulin and lupeol, two major compounds from the roots of *P. palatiferum*, demonstrated their selective inhibition against *Staphylococcus aureus* with an IC_{50} of 26.2 and 34.1 µM, respectively. Furthermore, lupeol inhibited selectively against MCF7 cells, while betulin showed inhibition of all three tested cancer cell lines, MCF7, HepG2, and KB.

Compound **1** was isolated as a light yellow crystal and optically active, $[\alpha]_D^{30}$ +28.2 (*c* 0.71, $CHCl_3$). The molecular formula of $C_{21}H_{20}O_8$ was determined from base peak at *m*/*z* 423.1064 $[M + Na]^+$ in the HR-ESI mass spectrum of **1**. Its IR spectrum suggested the presence of hydroxyl functionality (ν_{max} 3427 cm^{-1}). In the ^1H NMR spectrum, compound **1** displayed signals corresponding to a 1,3,4-trisubstituted benzene ring at δ_H = 6.87 (dd, *J* = 8.0 and 1.5 Hz), 6.83 (d, *J* = 8.0 Hz), 6.92 (d, *J* = 1.5 Hz) and a 1,2,3,4-tretrasubstituted benzene ring at δ_H = 6.96 (d, *J* = 8.5 Hz) and 6.52 (d, *J* = 8.5 Hz). Also present were signals assigned to a methoxy at δ_H = 4.01 (s), two methylendioxy groups at δ_H = 5.92 (d, *J* = 1.0 Hz) and 5.97 (s), three methines at δ_H = 5.07 (d, *J* = 5.0 Hz), 2.93 (m), and 4.79 (s), two methylenes at δ_H = 4.53 (dd, *J* = 9.5 and 8.5 Hz) and 3.94 (dd, *J* = 9.5 and 5.5 Hz) and at δ_H = 3.92 (d, *J* = 9.0 Hz) and 4.04 (d, *J* = 9.0 Hz). The ^{13}C and DEPT spectra exhibited the presence of seven aromatic quaternary carbons (δ_C = 126.5, 140.6, 136.2, 156.6, 129.4, 148.1, 147.8) and a sp^3 quaternary carbon at δ_C = 91.2. Based on ^1H-^1H coupling constant analysis, the assignment of three separated spin-spin coupling systems was achieved: H-5′-H-6′, H-7′-H-8′-H-9′, and H-5-H-6-H-2 (1,3,4-trisubstituted benzene ring). The spectral features of cou-

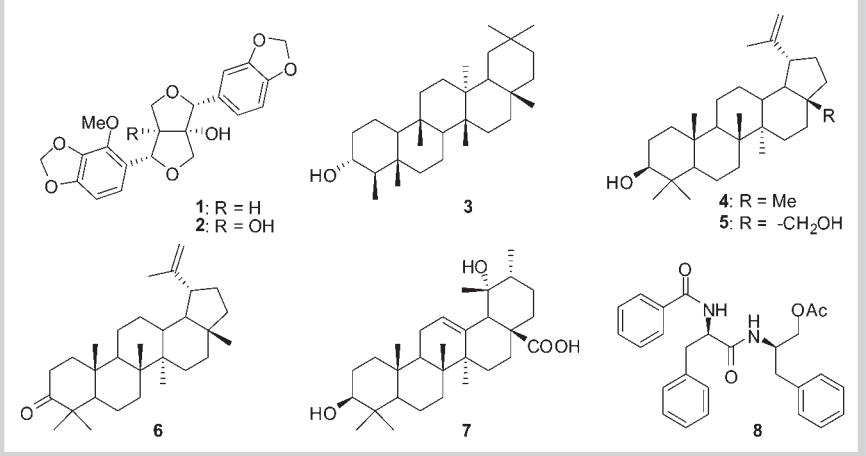

Fig. 1 Isolated compounds from the roots of *P. palatiferum*.

This is a copy of the author's personal reprint

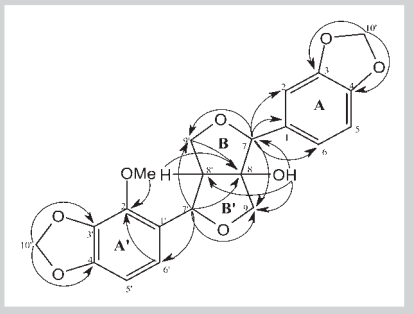

Fig. 2 Key HMBC correlations for **1**.

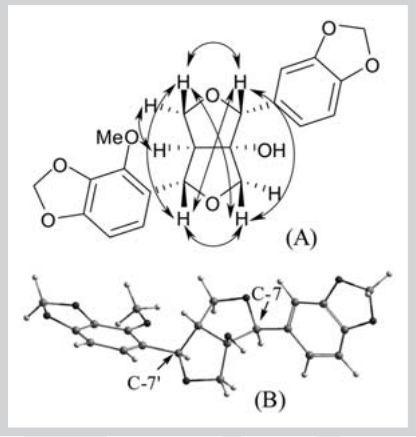

Fig. 3 Selected NOE interactions (**A**) and preferred conformation according to AM1 (**B**) for **1**.

pling systems were deduced from the HMBC experiment that indicated the structure of **1** to be a furanofuran lignan. The two methylenedioxy groups CH_2-10 (δ_C = 101.0, δ_H = 5.92) and CH_2-10′ (δ_C = 101.2, δ_H = 5.97) were determined to be linked to C-3 (δ_C = 148.1) and C-4 (δ_C = 147.8) and to C-3′ (δ_C = 136.2) and C-4′ (δ_C = 149.1) from their cross-peaks, respectively. Whereas the methoxy group (δ_C = 59.4, δ_H = 4.01) was bonded to C-2′ (δ_C = 140.6) as indicated by their correlations in the HMBC spectrum (**Fig. 2**). In the NOESY spectrum, H-9′*α* presented cross-peak with H-8′, while on the one hand, H-9*β* and H-9′*β* were correlated together, and on the other hand they displayed interactions with H-7 and H-7′. These data revealed a *trans* relationship between H-7′ and H-8′. In addition, the spatial interaction between H-7 and H-7′ indicated a *cis*-fused junction for B/B' rings and that these two protons were in pseudo-axial positions. This conclusion was supported by the coupling constant analysis of the benzylic proton H-7′ at δ_H 5.07 (J = 5.0 Hz) which suggested that the A′-ring had a pseudo-equatorial disposition [11,12]. The relative configuration of **1** was thus established as drawn in **Fig. 3**. This observation was in agreement with the lowest energy conformer (**Fig. 3B**) obtained by the AM1 method with the Hyper-Chem program (v. 8.0.3) [13] in which, the two aromatic rings had all pseudo-equatorial dispositions on the B- and B'-rings. The comparison of the optical rotation activity of **1** (+ 28.2, *c* 0.71, $CHCl_3$) with that of a compound with a similar structure, such as paulownin (+ 28.4, *c* 1.09, $CHCl_3$) with known absolute configuration [14,15], suggested the 7*S*,7′*R*,8*S*,8′*R* configuration for **1**. This compound was identified as 7*S*,7′*R*,8*S*,8′*R*-8-hydroxy-6-methoxy-3,4:3′,4′-bis(methylenedioxy)-7,9′:7′,9-diepoxylignan and named palatiferin A.

Compound **2** was obtained as yellow crystal and optically active, $[\alpha]_D^{30}$ − 53.0 (*c* 0.28, $CHCl_3$). The HR-ESI mass spectrum exhibited the base peak at *m/z* 439.1007 $[M + Na]^+$. 1D NMR spectra of **2** were close to those of **1** except for the presence of a sp^3 quaternary carbon at δ_C 88.2 (C-8) instead of the sp^3 methine group. The significant differences were noted for the proton signals of the furan fused rings. The typical chemical shifts of H-7 and CH_2-9 in compound **1** were at δ_H = 5.07 and δ_H = 4.53 and 3.94, respectively, whereas these values in **2** were at δ_H = 5.94 and δ_H = 4.07 and 4.28, respectively. The chemical shift of C-8′ (**Table 1**) suggested its linkage to an oxygen atom. Analysis of DEPT with the aid of 2D NMR spectra revealed the structure of **2**. The relative stereochemistry of this compound was then established by analysis of NOESY experiment in the same manner as **1** that indicated a relative configuration similarity of **1** to **2**: NOE interaction between H-7 and H-7′ was observed, indicating a *cis*-fused junction for two furan rings and two aromatic rings having pseudo-equatorial disposition. Compound **2** had a structural similarity and the same relative configuration when compared to wodeshiol which presented a negative value of optical rotation ($[\alpha]_D^{24}$ − 34.6, in $CHCl_3$) and had 7*S*,7′*S*,8*R* and 8′*R* configurations [16, 17]. As compound **2** also exhibited a negative $[\alpha]_D^{30}$ value (− 53.0, *c* 0.28, $CHCl_3$), the 7*S*,7′*S*,8*R*,8′*R* configurations were thus proposed for **2**. This compound was determined as 7*S*,7′*R*,8*R*,8′*R*-8,8′-dihydroxy-6-methoxy-3,4:3′,4′-bis(methylenedioxy)-7,9′:7′,9-diepoxylignan and named palatiferin B.

In the previous study [4], Huynh reported that the extract of *P. palatiferum* had an antimicrobial activity. Furthermore, this plant was used in Vietnam as a remedy for the treatment of cancer and cancer prevention. In order to identify its bioactive components, the major and the new compounds of this plant were tested for

Table 1 NMR data for **1** and **2** (^{1}H: 500.13 MHz, ^{13}C: 125.76 MHz, $CDCl_3$).

Position	1		2	
	δ_C	δ_H mult. (J in Hz)	δ_C	δ_H mult. (J in Hz)
1	129.4		130.1	
2	107.4	6.92 d (1.5)	107.8	6.91 br. s
3	148.1		147.8	
4	147.8		147.6	
5	108.5	6.83 d (8.0)	108.2	6.81 d (8.0)
6	120.1	6.87 dd (8.0, 1.5)	120.5	6.84 br. d (8.0)
7	86.9	4.79 (s)	86.0	4.91 s
8	91.2		88.1	
9	74.4	3.92 d (9.0, H*α*) 4.04 d (9.0, H*β*)	76.1	4.06 (m)
10	101.0	5.92 d (1.0)	101.1*	5.95 (s)
1′	126.5		121.3	
2′	140.6		140.5	
3′	136.2		136.3	
4′	149.1		149.7	
5′	102.1	6.52 d (8.5)	102.8	6.57 d (8.0)
6′	118.7	6.96 d (8.5)	120.3	6.94 d (8.0)
7′	82.4	5.07 d (5.0)	84.7	5.94 (s)
8′	60.2	2.93 (m)	88.2	
9′	72.7	4.53 dd (9.5, 8.5, H*α*) 3.94 dd (9.5, 5.5, H*β*)	77.0	4.07 d (10.0, H*α*) 4.28 d (10.0, H*β*)
10′	101.2	5.97 (s)	101.2*	5.95 (s)
OMe	59.4	4.01 (s)	59.6	4.03 (s)
8′-OH				2.65 (s)
8-OH		1.65 (s)		2.66 (s)

* Signals may be interchanged in each column

Table 2 Cytotoxicity (IC_{50} in μM) and antibacterial activity (IC_{50} in μM) of **1, 2, 5**, and **6.**

Target	1	2	5	6	Ellipticine	Ampicillin	Cefotaxime	Nystatin
MCF_7	> 150	> 150	42.9	15	1.6			
$HepG_2$	104.8	> 150	> 150	72.3	1.8			
KB	77.8	133	> 150	58.8	1.5			
E. coli	> 150	> 150	> 150	> 150			0.15	
P. aeruginosa	> 150	> 150	> 150	> 150			18.04	
C. albicans	> 150	> 150	> 150	> 150				1.94
B. subtilis	> 150	> 150	> 150	> 150		0.0062		
S. aureus	> 150	> 150	26.2	34.1		0.062		

their cytotoxicity and antimicrobial activity. The compounds **1, 2, 5**, and **6** were thus evaluated for their antimicrobial activity against *Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Bacillus subtilis*, and *Staphylococcus aureus*, and cytotoxicity against MCF7, HepG2, and KB cell lines. Lupeol and betulin had a selective inhibition against *S. aureus* with an IC_{50} of 26.2 and 34.1 μM, respectively. Palatiferin A (**1**) presented a moderate cytotoxicity against KB and HepG2 cells with IC_{50} values of 77.8 and 104.8 μM, respectively, whereas palatiferin B (**2**) showed a weaker inhibition. This observation suggested that the presence of the hydroxyl group at C-8′ of **2** decreased its cytotoxicity as compared to **1** (▶ **Table 2**). Betulin (**6**) was cytotoxic against all three tested cancer cell lines, while lupeol (**5**) displayed its selective inhibition against MCF7 cells with an IC_{50} of 42.9 μM. Many interesting biological properties, such as anticancer, antioxidant, and antimicrobial activities [18–26] have been reported for betulin and lupeol. Since betulin and lupeol are major compounds from the roots of *P. palatiferum*, triterpenoid and lignan compounds are probably the active components from this plant.

Materials and Methods

Plant material

The roots of *P. palatiferum* were collected at the garden of the 7-Nga Tay Ninh Company where the plant *P. palatiferum* is cultivated for making Hoan-Ngoc tea. A specimen (DVHAI 02) was identified by Msc. Do Van Hai and deposited at the herbarium of the Institute of Ecology and Natural Resources, Vietnam Academy of Science and Technology, Vietnam. The materials were dried in the drying room at 25 °C to obtain 1.7 kg. The plant materials were extracted with methanol and the residue of the extract was partitioned with *n*-hexane and EtOAc. The purification procedures of the *n*-hexane and EtOAc residues are detailed in the Supporting Information section.

Isolates

Palatiferin A (**1**): Light yellow crystal, mp 103–104 °C; R_f 0.62, silica gel 60 F_{254}, *n*-hexane/CH_2Cl_2/acetone (1/1/0.5); $[\alpha]_D^{30}$ + 28.2 (*c* 0.71, $CHCl_3$), UV (MeOH): λ_{max} (log ε) = 284 (3.2), 215 (3.8); IR (KBr): ν_{max} = 3427, 2926, 1631, 1471, 1344, 1262, 1034, 808, 560 cm^{-1}; positive EI-MS: *m/z* = 400 $[M]^+$; positive HR-EIS-MS: *m/z* = 423.1064 $[M + Na]^+$ (calcd. for $C_{21}H_{20}O_8Na$ 423.1056); NMR data see ▶ **Table 1**.

Palatiferin B (**2**): Light yellow crystal, mp 167–169 °C, R_f 0.49, silica gel 60 F_{254}, *n*-hexane/CH_2Cl_2/acetone (1/1/0.5); $[\alpha]_D^{30}$ − 53.0 (*c* 0.28, $CHCl_3$), UV (MeOH): λ_{max} (log ε) = 284 (3.6), 213 (4.3); IR (KBr): ν_{max} = 3446, 2884, 1629, 1495, 1440, 1243, 1041, 928, 778 cm^{-1}; positive EI-MS: *m/z* = 416 $[M]^+$; positive HR-EI-MS: *m/z* = 439.1007 $[M]^+$ (calcd. for $C_{21}H_{20}NaO_9$ 439.1005); NMR data see ▶ **Table 1**.

Antimicrobial activity

The bacterial strains used for the antimicrobial assay were cultures of *Escherichia coli* (ATTC 25922), *Pseudomonas aeruginosa* (ATTC 15442), *Candida albicans* (ATTC 10231), *Bacillus subtilis* (ATTC 6633), and *Staphylococcus aureus* (ATTC 13709), all obtained from the American Type Culture Collection (ATCC). The antimicrobial assay was performed as previously reported method [27] (see Supporting Information). The positive controls, ampicillin (purity > 98%), cefotaxime (purity ~ 95%), and nystatin (BioXtra, γ-irradiated) were purchased from Sigma.

Cytotoxic assay

Cytotoxicity of compounds **1, 2, 5**, and **6** (purity > 95%) against tumor cell lines was monitored, according to the previously described method [28] (see Supporting Information). The human epidermal carcinoma (KB) was obtained from the American Type Culture Collection (ATCC). The positive control, ellipticine (purity > 99%), was purchased from Sigma.

Supporting information

1D and 2D NMR spectra and HR-ESI-MS of compounds **1** and **2** and detailed protocols for the cytotoxicity and antimicrobial assays are available as Supporting Information.

Acknowledgements

We thank Dr. Dang Vu Luong and Msc. Ba Thi Cham (Institute of Chemistry in Hanoi) for NMR spectra and biological assays, respectively.

References

1 *Oanh LTL.* Investigation of some biochemical characters of proteolytic activity of *Pseuderanthemum palatiferum.* J Mater Med (Vietnamese) 1999; 4: 13–17

2 *Huynh KD, Chau BL, Yamasaki S, Hirara H.* The Effects of *Pseuderanthemum palatiferum*, a new medicinal plant, on growth performances and diarrhea of piglets. Jpn Agric Res Q 2006; 40: 85–90

3 *Phan MG, Ha VB, Phan TS.* Phytochemical investigation of *Pseuderanthemum palatiferum* (Nees) Radlk., (Acanthaceae). J Chem (Vietnamese) 2003; 2: 115–118

4 *Huynh KD.* Phytochemical study of the leaves of *Pseuderanthemum palatiferum.* J Sci (Vietnamese) 2008; 9: 232–240

5 *Kundu JK, Rouf ASS, Nazmul-Hossain MD, Hasan CM, Rashid MA.* Antitumor activity of epifriedelanol from *Vitis trifolia*. Fitoterapia 2000; 71: 577–579

6 *Moriarity DM, Huang J, Yancey CA, Zhang P, Setzer WN, Lawton RO, Bates RB, Caldera S.* Lupeol is the cytotoxic principle in the leaf extract of *Dendropanax* cf. *auerceti*. Planta Med 1998; 64: 370–372

7 *Siddiqui S, Hafeez F, Begum S, Siddiqui BS.* Oleanderol, a new pentacyclic triterpene from the leaves of *Nerium oleande.* J Nat Prod 1988; 51: 229–233

8 *Hisham A, Jaya-Kumar G, Fujimoto Y, Hara N.* Salacianone and salacianol, two triterpenes from *Salacia beddomei.* Phytochemistry 1995; 40: 1227–1231

9 *Numata A, Yang P, Takahashi C, Fujki R, Nabae M, Fujita E.* Cytotoxic triterpenes from a Chinese medicine, Goreishi. Chem Pharm Bull 1989; 37: 648–651

This is a copy of the author's personal reprint

10 *Ishiguro K, Nagata S, Fukumoto H, Yamaki M, Takagi S, Isoi K.* A dipeptide derivative from *Hypericum japonicum*. Phytochemistry 1991; 30: 3639–3641
11 *Pelter A, Ward RS.* General methods for the assignment of stereochemistry to 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane lignans. Tetrahedron 1976; 32: 2783–2788
12 *Kikuchi T, Matsuda S, Kadota S, Tai T.* Studies on the constituents of medicinal and related plants in Sri Lanka. III. Novel sesquilignan from *Hedyotis lawsoniae*. Chem Pharm Bull 1985; 33: 1444–1451
13 HyperChem manual. Gainsville, FL: Hypercube, Inc.; 2002
14 *Anjaneyulu ASR, Rao KJ, Rao VK, Row LR, Subrahmanyam C, Pelter A, Ward RS.* The structures of lignans from *Gmelina arborea* Linn. Tetrahedron 1975; 31: 1277–1285
15 *Okazaki M, Ishibashi F, Shuto Y, Taniguchi E.* Total synthesis of (+)-paulownin. Biosci Biotechnol Biochem 1997; 61: 743–745
16 *Anjaneyulu ASR, Ramaiah PA, Row LR, Pelter A, Ward RS.* The structure of wodeshiol – the first of a new series of lignans. Tetrahedron Lett 1975; 16: 2961–2964
17 *Han X, Corey EJ.* A catalytic enantioselective total synthesis of (−)-wodeshiol. Org Lett 1999; 1: 1871–1872
18 *Yamashita K, Lu H, Lu J, Chen G, Yokoyama T, Sagara Y, Manabe M, Kodama H.* Effect of three triterpenoids, lupeol, betulin, and betulinic acid on the stimulus-induced superoxide generation and tyrosyl phosphorylation of proteins in human neutrophils. Clin Chim Acta 2002; 325: 91–96
19 *Sporn MB, Suh N.* Chemoprevention of cancer. Carcinogenesis 2000; 21: 525–530
20 *Saleem M, Maddodi N, Abu-Zaid M, Khan N, Hafeez BB, Asim M, Suh Y, Yun JM, Setaluri V, Mukhtar H.* Lupeol inhibits growth of highly aggressive human metastatic melanoma cells *in vitro* and *in vivo* by inducing apoptosis. Clin Cancer Res 2008; 14: 2119–2127
21 *Murtaza I, Saleem M, Adhami VM, Hafeez BB, Mukhtar H.* Suppression of cFLIP by lupeol, a dietary triterpene, is sufficient to overcome resistance to TRAIL-mediated apoptosis in chemoresistant human pancreatic cancer cells. Cancer Res 2009; 69: 1156–1165
22 *Mukherjee PK, Sahoo AK, Narayanan N, Kumar NS, Ponnusankar S.* Lead finding from medicinal plants with hepatoprotective potentials. Expert Opin Drug Discov 2009; 4: 545–576
23 *Yamashita K, Lu H, Lu J, Chen G, Yokoyama T, Sagara Y, Manabe M, Kodama H.* Effect of three triterpenoids, lupeol, betulin, and betulinic acid on the stimulus-induced superoxide generation and tyrosyl phosphorylation of proteins in human neutrophils. Clin Chim Acta 2002; 325: 91–96
24 *Saleem M, Murtaza I, Tarapore RS, Suh Y, Adhami VM, Johnson JJ, Siddiqui IA, Khan N, Asim M, Hafeez BB, Shekhani MT, Li B, Mukhtar H.* Lupeol inhibits proliferation of human prostate cancer cells by targeting β-catenin signaling. Carcinogenesis 2009; 30: 808–817
25 *Zhang L, Zhang Y, Zhang L, Yang X, Lv Z.* Lupeol, a dietary triterpene, inhibited growth, and induced apoptosis through downregulation of DR3 in SMMC7721 cells. Cancer Invest 2009; 27: 163–170
26 *Alessia P, Gaetano P, Ugo T.* Triterpenoids as new promising anticancer drugs. Anticancer Drugs 2009; 20: 880–892
27 *Hadacek F, Greger H.* Testing of antifungal natural products: methodologies, comparability of results and assay choice. Phytochem Anal 2000; 11: 137–147
28 *Scudiero DA, Shoemaker RH, Kenneth DP, Monks A, Tierney S, Nofziger TH, Currens MJ, Seniff D, Boyd MR.* Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res 1988; 48: 4827–4833

received September 15, 2010
revised December 9, 2010
accepted December 11, 2010

Bibliography
DOI http://dx.doi.org/10.1055/s-0030-1250683
Published online January 17, 2011
Planta Med

ISSN 0032-0943

Correspondence
Dr. Huong Doan Thi Mai
Institute of Marine Biochemistry
Vietnam Academy of Science and Technology
18, Hoang Quoc Viet Road
Caugiay, Hanoi
Vietnam
Phone: + 84 4 37 56 93 51
Fax: + 84 4 38 36 12 83
dtmhuong@ich.vast.ac.vn

Dr. Van Cuong Pham
Institute of Marine Biochemistry
Vietnam Academy of Science and Technology
18, Hoang Quoc Viet Road
Caugiay, Hanoi
Vietnam
Phone: + 84 4 37 56 49 95
Fax: + 84 4 38 36 12 83
phamvc@ich.vast.ac.vn

This is a copy of the author's personal reprint