NPC Natural Product Communications

Composition and Antifungal Activities of the Leaf Essential Oil of *Neolitsea parvigemma* from Taiwan

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Received: March 31st, 2011; Accepted: May 12th, 2011

The hydrodistillated leaf essential oil of *Neolitsea parvigemma* was analyzed to determine its composition and yield. Sixty-two compounds were identified, the main components being β -caryophyllene (14.2%), β -eudesmol (12.9%), α -cadinol (10.2%) and τ -cadinol (8.8%). Oxygenated sesquiterpenes (48.9%) and sesquiterpene hydrocarbons (48.8%) were the predominant groups of compounds. The antifungal indexes of the leaf oil against the 7 fungi, *Aspergillus clavatus, A. niger, Chaetomium globosum, Cladosporium cladosporioides, Myrothecium verrucaria, Penicillium citrinum* and *Trichoderma viride*, were 100.0, 72.3, 100.0, 100.0, 100.0, 75.8 and 88.6% at a 1 mg/mL concentration, respectively. The oil also exhibited anti-wood-decay-fungi activity against *Trametes versicolor, Phaneochaete chrysosporium, Phaeolus schweintizii*, and *Lenzites sulphureu* with MIC values of 50, 50, 25 and 25 µg/mL, respectively. For the antifungal and anti-wood-decay fungal activities of the oil, the active source compounds were determined to be α -cadinol, β -eudesmol and τ -cadinol.

Keywords: Neolitsea parvigemma, Lauraceae, essential oil, antifungal activity, anti-wood-decay fungal activity, β-caryophyllene, β-eudesmol.

Neolitsea parvigemma Kan and Sas (Lauraceae) is a small tree growing in the central and southern mountainous regions in Taiwan [1]. Previous reports suggested that the extractive exhibited bioactivities, such as antiplatelet aggregation, anti-inflammatory and cytotoxicity [2-6]. There are no literature reports on the chemical composition and biological activities of the essential oil from this species. Therefore, we used hydrodistillation to collect the leaf oil, which was analyzed by GC/FID and GC/MS.

The climate of Taiwan is warm and humid, and thus conducive to the growth of mildew. Mildew growth causes problems in the preservation of cultural items as induces allergies, well as asthma, bronchitis, cerebral infections, onychomycosis, pneumonia, peritonitis, and immune-deficiency syndrome [7]. We also applied the essential oil to seven strains of mold fungi and four strains of wood decay fungi to examine the interdiction efficacies. The second part of the study examined the antifungal activities of the leaf oil. The purpose of this study was to establish a chemical basis for the effective multipurpose utilization of the species.

Hydrodistillation of *N. parvigemma* leaves gave a yellowcolored oil with a yield of 1.08 ± 0.05 mL/100 g, based on the dry weight of leaves. The identified constituents are presented in Table 1, where all compounds are listed in

order of their elution from the DB-5 column. Sixty-two components were identified, representing 100% of the oil. groups, Among the oxygenated sesquiterpenes (48.9%), predominated followed by, sesquiterpene hydrocarbons (48.8%), non-terpenoids (1.5%),monoterpene hydrocarbons (0.5%) and oxygenated (0.3%). monoterpenes Among the oxygenated sesquiterpenes, β -eudesmol (12.9%), α -cadinol (10.2%) and τ -cadinol (8.8%) were the major compounds. Of the sesquiterpene hydrocarbons, β-caryophyllene (14.2%) was the main components.

<u>2011</u>

Vol. 6 No. 9 1357 - 1360

Although the leaf oil constituents of *N. parvigemma* was primarily sesquiterpenoids, like those of *N. pallens* [8], *N. australiensis*, *N. brassii*, *N. dealbata* [9,10], *N. sericea* [11], *N. foliosa* var. *caesia* [12] and *N. fischeri* [13], their main components differed. Further comparison with the leaf oil of *N. oblongifolia* and *N. umbrosa* [14] were predominantly monoterpenoids and differed from the leaf oil of *N. parvigemma*.

The antifungal indexes of the leaf oil against the 7 fungi, Aspergillus clavatus (A. c.), A. niger (A. n.), Chaetomium globosum (Ch. g.), Cladosporium cladosporioides (Cl. c.), Myrothecium verrucaria (M. v.), Penicillium citrinum (P. c.) and Trichoderma viride (T. v.), were 100.0, 72.3, 100.0, 100.0, 100.0, 75.8 and 88.6% at a 1 mg/mL concentration, respectively (Table 2). These results showed that leaf oil

Table 1: Chemical composition of the leaf oil N. parvigemma.

Compound ID	RI ª	Conc. (%)	Identification ^o
α-Pinene	939	t ^c	MS, KI, ST
Sabinene	975	ť	MS, KI, ST
β-Pinene	979	0.4	MS, KI, ST
(E)-β-Ocimene	1050	0.1	MS, KI, ST
Linalool	1097	0.3	MS, KI, ST
Terpinen-4-ol	1177	t	MS, KI, ST
(Z)-3-Hexenyl Butyrate	1184	0.1	MS, KI
Hexanoic acid, butyl ester	1186 1189	t	MS, KI, ST
α-Terpineol 2,3-Dimethyl benzofuran	1222	t 0.1	MS, KI, ST MS, KI
2-Prenyl cyclopentanone	1222	t	MS, KI
(3Z)-Hexenyl 2-methyl butanoate	1232	0.3	MS, KI
(3Z)-Hexenyl 3-methyl butanoate	1235	t	MS, KI
(2E)-Decenal	1263	t	MS, KI, ST
(3Z)-Hexenyl valerate	1281	t	MS, KI
(3Z)-Hexenyl tiglate	1321	t	MS, KI
δ-Elemene	1338	0.2	MS, KI
α-Cubebene	1351 1375	0.1	MS, KI MS, KI MS, KI MS, KI MS, KI, ST
α-Ylangene α-Copaene	1373	t 0.1	MS KI ST
(<i>3Z</i>)-Hexenyl hexanoate	1384	0.8	MS, KI, ST
β-Elemene	1391	3.3	MS, KI
iso-Caryophyllene	1409	0.1	MS, KI MS, KI MS, KI
α-Cedrene	1412	0.1	MS, KI
β-Caryophyllene	1419	14.2	MS, KI, ST
β-Gurjunene	1434	0.1	MS, KI
β-Copaene	1432	0.1	MS, KI
α-Gurjunene Aromadendrene	$1440 \\ 1441$	0.2 0.1	MS, KI MS, KI, ST
Aromadendrene trans-Muurola-3,5-diene	1454	0.2	MS, KI
(Z) - β -Farnesene	1457	3.5	MS, KI, ST
allo-Aromadendrene	1460	2.9	MS, KI, ST
9-epi-(E)-Caryophyllene	1466	0.1	MS, KI
trans-Cadina-1(6),4-diene	1477	0.3	MS, KI
γ-Gurjunene	1477	0.4	MS, KI, ST
γ-Muurolene Valencene	1480 1496	4.3 0.5	MS, KI
Varidiflorene	1490	0.5	MS, KI MS, KI
Bicyclogermacrene	1500	4.5	MS, KI
α-Muurolene	1500	4.0	MS, KI
Epizonarene	1502	0.4	MS, KI
α-Bulesene	1510	1.0	MS, KI
γ-Cadinene	1514	0.4	MS, KI MS, KI MS, KI MS, KI MS, KI MS, KI
δ-Cadinene	1523	4.7	MS, KI
Zonarene	1530 1535	0.2 0.4	MS, KI MS, KI
<i>trans</i> -Cadina-1(2),4-diene α-Cadinene	1535	0.4	MS, KI
α-Calacorene	1546	0.1	MS, KI
Selina-3,7(11)-diene	1547	0.1	MS, KI
Elemol	1550	0.8	MS, KI
Germacrene B	1561	0.6	MS, KI
trans-Nerolidol	1563	1.7	MS, KI, ST
Spathulenol	1578	1.8	MS, KI
Caryophyllene oxide Globulol	1583 1585	4.3 1.2	MS, KI, ST MS, KI, ST
Viridiflorol	1593	1.2	MS, KI
1-epi-Cubenol	1629	1.7	MS, KI
γ-Eudesmol	1632	4.3	MS, KI
τ-Cadinol	1640	8.8	MS, KI, ST
β-Eudesmol	1651	12.9	MS, KI, ST
α -Cadinol	1654	10.2	MS, KI, ST
14-hydroxy-9- <i>epi-(E)</i> -Caryophyllene	1669	1.3	MS, KI
Compound identified		0.5	
Monoterpene hydrocarbon Monoterpene orvgen		0.5 0.3	
Monoterpene oxygen Sesquiterpene hydrocarbon		48.8	
Sesquiterpene nyurocaroon Sesquiterpene oxygen		48.9	
Others		1.5	
Oil yield (mL/100g)		1.08 ± 0.05	
^a Detention index on a DD 5 column	with rof		llroman [15]

^a Retention index on a DB-5 column with reference to *n*-alkanes [15]. ^b MS, NIST and Wiley library spectra and the literature; RI, Retention index; ST, authentic standard compounds. ^c.trace < 0.1%

was totally inhibitory to mycelial growth of *A. clavatus, Cl. cladosporioides, Ch. globosum,* and *M. verrucaria* among the fungi tested. Comparing with the antifungal activities of the essential oils from *Eucalyptus urophylla, E. grandis, E. camaldulensis, E. citriodora* [7], *Litsea cubeba* [16] and *L. coreana* [17], the leaf oil of *N. parvigemma* was superior (Table 2). The results verified that *N. parvigemma* leaf oil has excellent antifungal activities.

Table 2: Comparison of the antifungal index (%) of the leaf oils (1 mg/mL) of *N. parvigemma* and theose of *Eucalyptus urophylla*, *E. grandis*, *E. camaldulensis*, *E. citriodora*, *Litsea cubeba* and *L. coreana* against the fungi.

Essential oil	Fungi ^a						- Reference	
Essential off	А. с.	A. n.	Cl. c.	Ch. g.	<i>M</i> . <i>v</i> .	Р. с.	<i>T. v</i> .	Reference
Neolitsea parvigemma	100.0	72.3	100.0	100.0	100.0	75.8	88.6	This study
Eucalyptus urophylla	2.4	0.0	12.6	100.0	30.7	12.3	0.0	[7]
E. grandis	56.5	16.1	38.2	100.0	54.8	8.6	0.0	[7]
E. camaldulensis	3.9	0.0	22.2	0.0	26.5	14.3	0.0	[7]
E. citriodora	28.2	0.0	38.4	100.0	100.0	26.4	0.0	[7]
Litsea cubeba	100.0	56.2	100.0	95.3	100.0	58.3	68.6	[16]
L. coreana	89.0	49.0	95.7	78.3	86.0	43.3	52.7	[17]

Note: ^a A. c.: Aspergillus clavatus; A. n.: A. niger; Cl. c.: Cladosporium cladosporioides; Ch. g.: Chaetomium globosum; M. v.: Myrothecium verrucaria; P. c.: Penicillium citrinum; T. v.: Trichoderma viride

Table 3: IC₅₀ values (μ g/mL) of the five main constituents of *N*. *parvigemma* leaf oil against 7 fungi strain.

Constituents a	Fungi ^b						
Constituents	А. с.	A. n.	СІ. с.	Ch.g.	<i>M</i> . <i>v</i> .	Р. с.	<i>T. v</i> .
Neolitsea parvigemma (Crude leaf oil)	56.8	92.3	36.8	80.5	68.8	138.2	100.8
β-caryophyllene	92.3	95.2	48.1	100.6	90.8	190.3	160.8
a-cadinol	21.0	45.6	13.1	36.8	18.1	55.8	48.6
τ -cadinol	28.3	66.8	20.8	52.3	43.5	66.9	68.6
β-eudesmol	23.0	53.6	16.3	43.6	38.6	59.8	51.3
Nystatine ^c	<12.5	<12.5	<12.5	<12.5	<12.5	<12.5	<12.5

^a1. β-caryophyllene (\geq 98.5%), 2. α-cadinol (100%), 3. τ-cadinol (100%), 4. β-eudesmol (\geq 98%). Compound 1 was purchased from the Fluka Co. (Milwaukee, USA), and Compound 4 was purchased from the Wako Co. (Tokyo, Japan), where as the Compound 2 and 3 were from isolate of the Ho et al. [19] study on *Machilus philippinenesis* essential oil.

^b A. c.: Aspergillus clavatus; A. n.: A. niger; Cl. c.: Cladosporium cladosporioides; Ch. g.: Chaetomium globosum; M. v.: Myrothecium verrucaria; P. c.: Penicillium citrinum; T. v.: Trichoderma viride °Nystatine is an antifungal drug and used as a positive control.

However, in order to ascertain the source compounds of antifungal activities from N. parvigemma, the main components were individually tested for their antifungal activities. The sesquiterpenoids, β -caryophyllene, α -cadinol, β -eudesmol and τ -cadinol, exhibited better activities among the leaf oil constituents, in particular, α -cadinol, β -eudesmol and τ -cadinol. α -Cadinol, β -eudesmol and τ -cadinol exhibited strong activity against A. clavatus, Cl. cladosporioides, Ch. globosum and M. verrucaria with the highest antifungal indexes ranging from 82 to 100% at 100 μ g/mL. IC₅₀ values of α -cadinol, against A. clavatus, Cl. cladosporioides, Ch. globosum and M. verrucaria were 21.0, 13.1, 36.8, and 18.1 μ g/mL, for β -eudesmol, 23.0, 16.3, 43.6, and 38.6 μ g/mL, and for τ -cadinol, 28.3, 20.8, 52.3 and 43.5 μ g/mL (Table 3). These results indicated that the active source compounds were α -cadinol, β -eudesmol and τ -cadinol, which supports previous work showing high antimicrobial activity of these compounds [18].

The essential oil of *N. parvigemma* was tested against 2 white rot fungi (*Trametes versicolor, Phanerochaete chrysosporium*) and 2 brown rot fungi (*Phaeolus schweinitzii, Lenzites sulphureu*). The anti-wood-decay fungal indices presented in Table 4 are a clear demonstration of the excellent anti-wood-decay fungal property of the oil. The growth of *T. versicolor, Phane. chrysosporium, Phaeo. schweinitzii* and *L. sulphureu* was completely inhibited at concentrations of 50, 50, 25 and

Table 4: Anti-wood-decay fungal indices of leaf essential oil from N. parvigemma.

	Anti-wood-decay fungal index (%)					
Dosage (µg/mL)	Trametes versicolor	Phaneochaete chrysosporium	Phaeolus schweintizii	Lenzites sulphureu		
Essential oil						
12.5	48 ± 3.3	36 ± 3.3	52 ± 6.6	68 ± 3.3		
25	82 ± 6.6	68 ± 6.6	100 ± 3.3	100 ± 0		
50	100 ± 0	100 ± 0	100 ± 0	100 ± 0		
75	100 ± 0	100 ± 0	100 ± 0	100 ± 0		
100	100 ± 0	100 ± 0	100 ± 0	100 ± 0		
DDAC						
12.5	100 ± 0	100 ± 0	100 ± 0	100 ± 0		

Note: DDAC (didecyl dimethyl ammonium chloride) is a wood preservative for wood decay fungi and used as a positive control.

Table 5: Comparison of the MIC values (μ g/mL) of the leaf oils of *N*. *parvigemma* and those of *Machilus philippinensis*, *M. pseudolongifolia*, *M. kusanoi* and *L. coreana* against the wood-decay fungi.

Essential oil		Fungi ^a				
	<i>T. v</i> .	Phane. c.	Phaeo. s.	L. s.	- Reference	
Neolitsea parvigemma	50	50	25	25	This study	
Machilus philippinensis	100	100	100	50	[19]	
M. pseudolongifolia	75	75	75	25	[20]	
M. kusanoi	75	75	75	25	[21]	
Litsea coreana	75	75	50	25	[17]	

Note: ^a T. v.: Trametes versicolor; Phane. c.: Phaneochaete chrysosporium; Phaeo. s: Phaeolus schweintizii; L. s.: Lenzites sulphureu

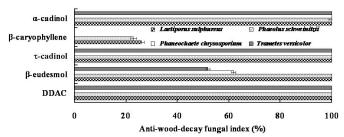


Figure 1: Anti-wood-decay fungal indices of the four main compounds $(50 \ \mu\text{g/mL})$ of the leaf essential oil of *N. parvigemma* Note: DDAC (didecyl dimethyl ammonium chloride) is a wood preservative for wood decay fungi and used as a positive control.

25 µg/mL, respectively (Table 4). Comparing the antiwood-decay fungal activities of the essential oils from *Machilus philippinensis* [19], *M. pseudolongifolia* [20], *M. kusanoi* [21] and *L. coreana* [17] the leaf oil of *N. parvigemma* was superior (Table 5). The results verified that *N. parvigemma* leaf oil has excellent anti-wood-decay fungal activities.

Furthermore, in order to ascertain the source compounds of the *N. parvigemma* essential oil, we also tested the anti-wood-decay fungal activities of its major component compounds (Figure 1). The results indicated that the sources of activities were also α -cadinol, β -eudesmol and τ -cadinol. At a 50 µg/mL concentration, α -cadinol and τ -cadinol showed total growth inhibition against all whiterot and brown-rot fungi tested; while β -eudesmol at 50 µg/mL concentration could completely inhibit brown-rot fungi but partially inhibit white-rot fungi. The results agree with those of Kondo and Imamura [22] and Chang *et al.* [23]. Thus, the excellent wood-decay-fungi inhibitive activities exhibited by the *N. parvigemma* leaf oil could well be contributed by the presence of compounds such as α -cadinol, β -eudesmol and τ -cadinol etc.

Experimental

Plant materials: Fresh leaves of *N. parvigemma* were collected in July 2009 from the Dahanshan at an elevation of 1800 m in southern Taiwan (N 22° 26′ 38″, E 120° 42′ 36″, Pingtung County). The samples were compared with specimen no. ou 6889 from the Herbarium of National Chung-Hsing University and positively identified by Prof. Yen-Hsueh Tseng of NCHU. The voucher specimen (CLH-011) has been deposited in the NCHU herbarium. Leaves of the species were collected for subsequent extraction and analysis.

Isolation of the leaf essential oil: Leaves of *N. parvigemma* (1Kg) were placed in a round-bottom flask and hydrodistilled for 8 h with 3 L of distilled water. The essential oil removed was dried with anhydrous sodium sulfate. The oil yield and all test data are the average of triplicate analyses.

GC/FID analysis: A Hewlett-Packard HP 6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 μ m film thickness, J&W Scientific) and a FID detector was used for the quantitative determination of oil components. Oven temperature was programmed as follows: 50°C for 2 min, rising to 250°C at 5°C/min. Injector temperature: 270°C. Carrier gas: He with a flow rate of 1 mL/min. Detector temperature: 250°C, split ratio: 1:10. Diluted samples (1.0 μ L, 1/100, v/v, in ethyl acetate) were injected manually in the split mode. All data were the average of triplicate analyses.

GC/MS analysis: GC/MS analyses of the oil was performed by split injection of 1.0 μ L, of the oil on a Hewlett-Packard HP 6890 gas chromatograph fitted with DB-5 fused silica capillary column (30 m x 0.25 mm x 0.25 μ m film thickness, J&W Scientific) coupled with a model 5973 mass detector. GC/MS operation conditions: injector temperature 270°C; oven temperature program as follows: 50°C for 2 min, rising to 250°C at 5°C/min; carrier gas: He with a flow rate of 1 mL/min, split ratio: 1:10. Mass spectra: Electron Impact (El⁺) mode 70 eV with a mass range of 30 to 450 *m/z*, ion source temperature 280°C. All data were the average of triplicate analyses.

Component identification: Identification of the leaf essential oil constituents was based on comparisons of retention index (RI) [15], retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra, and literature [15].

Antifungal assays: The mold fungi and wood decay fungi were obtained from the Culture Collection and Research Center of the Food Industry Research and Development Institute, Hsinchu City, Taiwan. For the mold fungi strains, references of ASTM G21, JIS Z 2911 and AATCC test method 30 were consulted and 7 strains including *A. clavatus* (ATCC 1007), *A. niger* (ATCC 6275), *Ch.* globosum (ATCC 6205), *Cl. cladosporioides* (ATCC 13276), *M. verrucaria* (ATCC 9095), *P. citrinum* (ATCC 9849), *T. viride* (ATCC8678) were tested. For the wood decay fungi were *T. versicolor* (BCRC 35253), *Phane. chrysosporium* (BCRC 36200), *Phaeo. schweinitzii* (Fries) (BCRC 35365) and *L. sulphureus* (BCRC 35305). Antifungal assays were and its main constituents were dissolved in 150 μ L of ethanol, respectively, and then added into 15 mL PDA to obtain the carried out in triplicate and the data were averaged. Leaf oil different

final concentrations. The test plates were incubated at 27°C. When the mycelium of fungi reached the edge of the control plate, the antifungal index was calculated as follows: Anti-fungal index (%)= (1–Da/Db) X 100, where Da is the diameter of the growth zone in the experimental dish (cm) and Db is the diameter of the growth zone in the control dish (cm). IC₅₀ (concentration that produces a 50% inhibitory effect) values of constituents were graphically obtained from the dose-response curves based on measurement at five different concentrations.

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Determination of Caffeoyl Quinic Acids and Flavonoids in <i>Acanthopanax trifoliatus</i> Leaves by HPLC Pongtip Sithisarn, Sarinthip Muensaen and Siripen Jarikasem	1289
Amides and Esters of Phenylpropenoic Acids from the Aerial Parts of <i>Trifolium pallidum</i>	
Barbara Szajwaj, Jaroslaw Moldoch, Milena Masullo, Sonia Piacente, Wieslaw Oleszek and Anna Stochmal Amides from the Stems of <i>Cinnamomum burmannii</i>	1293
Zi-Ling Hong, Jin-Cherng Huang, Soong-Yu Kuo and Chung-Yi Chen	1297
Phenolic Composition, Antioxidant Capacity and Antibacterial Activity of Selected Irish Brassica Vegetables Amit Kumar Jaiswal, Gaurav Rajauria, Nissreen Abu-Ghannam and Shilpi Gupta	1299
Dietary Burden of Phenolics Per Serving of "Mountain Tea" (<i>Sideritis</i>) from Macedonia and Correlation to Antioxidant Activity	
Jasmina Petreska, Marina Stefova, Federico Ferreres, Diego. A. Moreno, Francisco. A. Tomás-Barberán, Gjose Stefkov, Svetlana Kulevanova and Angel Gil-Izquierdo	1305
Aqueous Extract from <i>Vitis vinifera</i> Tendrils is Able to Enrich Keratinocyte Antioxidant Defences Daniele Fraternale, Roberta De Bellis, Cinzia Calcabrini, Lucia Potenza, Luigi Cucchiarini, Umberto Mancini, Marina Dachà and Donata Ricci	1315
A New Acylated Neohesperidoside from <i>Geranium purpureum</i>	1221
Didem Şöhretoğlu, Tibor Liptaj, M. Koray Sakar and Olov Sterner Synthesis and Field Test of Three Candidates for Soybean Pod Borer's Sex Pheromone	1321
Tao Zhang, Juntao Feng, Chonglin Cai and Xing Zhang	1323
Chemical Investigation of Carrageenan from the Red alga <i>Sarconema filiforme</i> (Gigartinales, Rhodophyta) of Indian Waters	
Sanjay Kumar, Gaurav K Mehta, Kamalesh Prasad, Ramavtar Meena and Arup K Siddhanta	1327
Monitoring the Emission of Volatile Organic Compounds from Flowers of <i>Jasminum sambac</i> Using Solid-Phase Micro-extraction Fibers and Gas Chromatography with Mass Spectrometry Detection VPPalayam Shanmugam Pragadheesh, Anju Yadav, Chandan Singh Chanotiya, Prasanta Kumar Rout and	
Girish Chandra Uniyal Valatile Commonante from Aprial ports of Contaurog gradilante and Conving son beganning Crowing Wild	1333
Volatile Components from Aerial parts of <i>Centaurea gracilenta</i> and <i>C. ovina</i> ssp. <i>besserana</i> Growing Wild n Bulgaria	
Carmen Formisano, Daniela Rigano, Felice Senatore, Svetlana Bancheva, Maurizio Bruno, Antonella Maggio and Jergio Rosselli	1339
V ariability of Essential Oils of <i>Betonica officinalis</i> (Lamiaceae) from Different Wild Populations in Kosovo Avni Hajdari, Behxhet Mustafa, Chlodwig Franz and Johannes Novak	1343
Analysis of Essential Oils from <i>Scutellaria orientalis</i> ssp. <i>alpina</i> and <i>S. utriculata</i> by GC and GC-MS Carmen Formisano, Daniela Rigano, Felice Senatore, Franco Piozzi and Nelly Apostolides Arnold	1347
Antibacterial Activity and GC/MS Analysis of the Essential Oils from Flower, Leaf and Stem of <i>Origanum vulgare</i> ssp. <i>viride</i> Growing Wild in North-west Iran Ali Shafaghat	1351
C omposition of <i>Satureja kitaibelii</i> Essential Oil and its Antimicrobial Activity Fatjana Kundaković, Marina Milenković, Saša Zlatković, Nada Kovačević and Nikolić Goran	1353
Composition and Antifungal Activities of the Leaf Essential Oil of <i>Neolitsea parvigemma</i> from Taiwan Chen-Lung Ho, Pei-Chun Liao, Eugene I-Chen Wang and Yu-Chang Su	1357
Antioxidant Capacity and Larvicidal and Antifungal Activities of Essential Oils and Extracts from <i>Piper krukoffii</i> Joyce Kelly R. da Silva, Eloisa Helena A. Andrade, Massuo J. Kato, Léa Maria M. Carreira, Elsie F Guimarães and	
José Guilherme S. Maia Evoluation of <i>Clausana anisata</i> Essential Oil from Comproon for Controlling Food Spoilage Europi and its	1361
Evaluation of <i>Clausena anisata</i> Essential Oil from Cameroon for Controlling Food Spoilage Fungi and its Potential Use as an Antiradical Agent	
Aoudou Yaouba, Léopold Ngoune Tatsadjieu, Pierre Michel Jazet Dongmo, François Xavier Etoa, Carl Moses Fontum Mbofung, Paul Henri Amvam Zollo and Chantal Menut	1367
Chemical Diversity in <i>Mentha spicata</i> : Antioxidant and Potato Sprout Inhibition Activity of its Essential Oils Shailendra S. Chauhan, Om Prakash, Rajendra C. Padalia, Vivekanand, Anil K. Pant and Chandra S. Mathela	1373
Role of Direct Bioautographic Method for Detection of Antistaphylococcal Activity of Essential Oils Györgyi Horváth, Noémi Jámbor, Erika Kocsis, Andrea Böszörményi, Éva Lemberkovics, Éva Héthelyi,	
Krisztina Kovács and Béla Kocsis	1379
Antiphytoviral Activity of Essential Oil from Endemic Species <i>Teucrium arduini</i> Valerija Dunkić, Nada Bezić and Elma Vuko	1385
Foxic Effects of <i>Citrus aurantium</i> and <i>C. limon</i> Essential Oils on <i>Spodoptera frugiperda</i> (Lepidoptera: Noctuidae) Emilio Villafañe, Diego Tolosa, Alicia Bardón and Adriana Neske	1389
Neutralizing Effects of <i>Nectandra angustifolia</i> Extracts against <i>Bothrops neuwiedi</i> Snake Venom Ana M. Torres, Francisco J. Camargo, Gabriela A. L. Ricciardi, Armando I. A. Ricciardi and Eduardo Dellacassa	1393
Ana W. Forres, Francisco J. Camargo, Gaoriera A. E. Kicchardi, Armando I. A. Kicchardi and Eduardo Denacassa Artocarpus Plants as a Potential Source of Skin Whitening Agents Enos Tangke Arung, Kuniyoshi Shimizu and Ryuichiro Kondo	1393
Mining Invertebrate Natural Products for Future Therapeutic Treasure	1071
Youmie Park	1403

Natural Product Communications 2011

Volume 6, Number 9

Contents

Original Paper	<u>Page</u>
Analysis of Car-3-en-5-hydroperoxide Nicole Lehnert, Ulrich Krings and Ralf G. Berger	1217
Antibacterial Potential of Citral Derivatives Soni A. Singh, Yogesh A. Potdar, Rasika S. Pawar and Sujata V. Bhat	1221
A New Bisabolene from <i>Stevia tomentosa</i> Alejandro Valdez-Calderón, J. Martín Torres-Valencia, J. Jesús Manríquez-Torres, René Velázquez-Jiménez, Mario A. Gómez-Hurtado, Luisa U. Román-Marín, Juan D. Hernández-Hernández, Carlos M. Cerda-García-Rojas and Pedro Joseph-Nathan	1225
Free Radical Scavenging Activity-Guided Isolation of a Diterpenoid from <i>Plectranthus punuctatus</i> O Wossen Kebede, Daniel Bisrat and Kaleab Asres	1229
Components from the Steamed Leaves of <i>Acanthopanax koreanum</i> and their Effects on PPAR Activity in HepG2 Cells Jeong Ah Kim, Seok Bean Song, Seo Young Yang and Young Ho Kim	1233
Isolation and X-ray Structure of Deoxycholic Acid from the Sponge Ircinia sp. Keisham Sarjit Singh and Werner Kaminsky	1237
Chemical Constituents of the Gorgonian Dichotella fragilis (Ridleg) from the South China Sea Yuan-Ming Zhou, Chang-Lun Shao, Chang-Yun Wang, Hui Huang, Ying Xu and Pei-Yuan Qian	1239
A New Pyrrolidine Derivative and Steroids from an Algicolous <i>Gibberella zeae</i> Strain Xiang-Hong Liu, Xiao-Zhen Tang, Feng-Ping Miao and Nai-Yun Ji	1243
Szentiamide, an <i>N</i> -formylated Cyclic Depsipeptide from <i>Xenorhabdus szentirmaii</i> DSM 16338 ^T Birgit Ohlendorf, Sven Simon, Jutta Wiese and Johannes F. Imhoff	1247
Bioactive Constituents from Michelia champaca Yu-Ting Yeh, Jin-Cherng Huang, Po-Lin Kuo and Chung-Yi Chen	1251
Inhibition of Gastric H ⁺ , K ⁺ -ATPase Activity by Compounds from Medicinal Plants Cristina Setim Freitas, Cristiane Hatsuko Baggio, Bárbara Mayer, Ana Cristina dos Santos, André Twardowschy, Cid Aimbiré de Moraes Santos and Maria Consuelo Andrade Marques	1253
GC/MS Analysis of Three Amaryllidaceae Species and Their Cholinesterase Activity Lucie Cahlíková, Nina Benešová, Kateřina Macáková, Klára Urbanová and Lubomír Opletal	1255
Astrotricoumarin, an antiproliferative 4'-hydroxy-2',3'-dihydroprenylated methylcoumarin from an Astrotrichilia sp. from the Madagascar dry forest Liva Harinantenaina, Peggy J. Brodie, Martin W. Callmander, Richard Randrianaivo, Stephan Rakotonandrasana, Vincent E. Rasamison, Etienne Rakotobe and David G. I. Kingston	1259
A New Chromene Isolated from Ageratum conyzoides Abiodun Humphrey Adebayo, Chang-Jiu Ji, Yu-Mei Zhang, Wen-Jun He, Guang-Zhi Zeng, Hong-Jin Han, Jun-Ju Xu, Afolabi Akintunde Akindahunsi and Ning-Hua Tan	1263
Antifibrotic Constituents from Garcinia mangostana Young-Won Chin, Eunjin Shin, Bang Yeon Hwang and Mi Kyeong Lee	1267
Antioxidant and Antimutagenic Polyisoprenylated Benzophenones and Xanthones from <i>Rheedia acuminata</i> Giovanna R. Almanza, Raúl Quispe, Patricia Mollinedo, Gloria Rodrigo, Odette Fukushima, Rodrigo Villagomez, Bjorn Akesson and Olov Sterner	1269
Anthraquinone Profile, Antioxidant and Antimicrobial Properties of Bark Extracts of <i>Rhamnus catharticus</i> and <i>R. orbiculatus</i> Marcello Locatelli, Francesco Epifano, Salvatore Genovese, Giuseppe Carlucci, Marijana Zovko Končić, Ivan Kosalec and Dario Kremer	1275
Quantitative Analysis of Euglobals in <i>Eucalyptus loxophleba</i> Leaves by qNMR Jasmeen Sidana, William J. Foley and Inder Pal Singh	1281
Evaluation of Antioxidant Activity of Isoferulic Acid in vitro Xiaozhen Wang, Xican Li and Dongfeng Chen	1285