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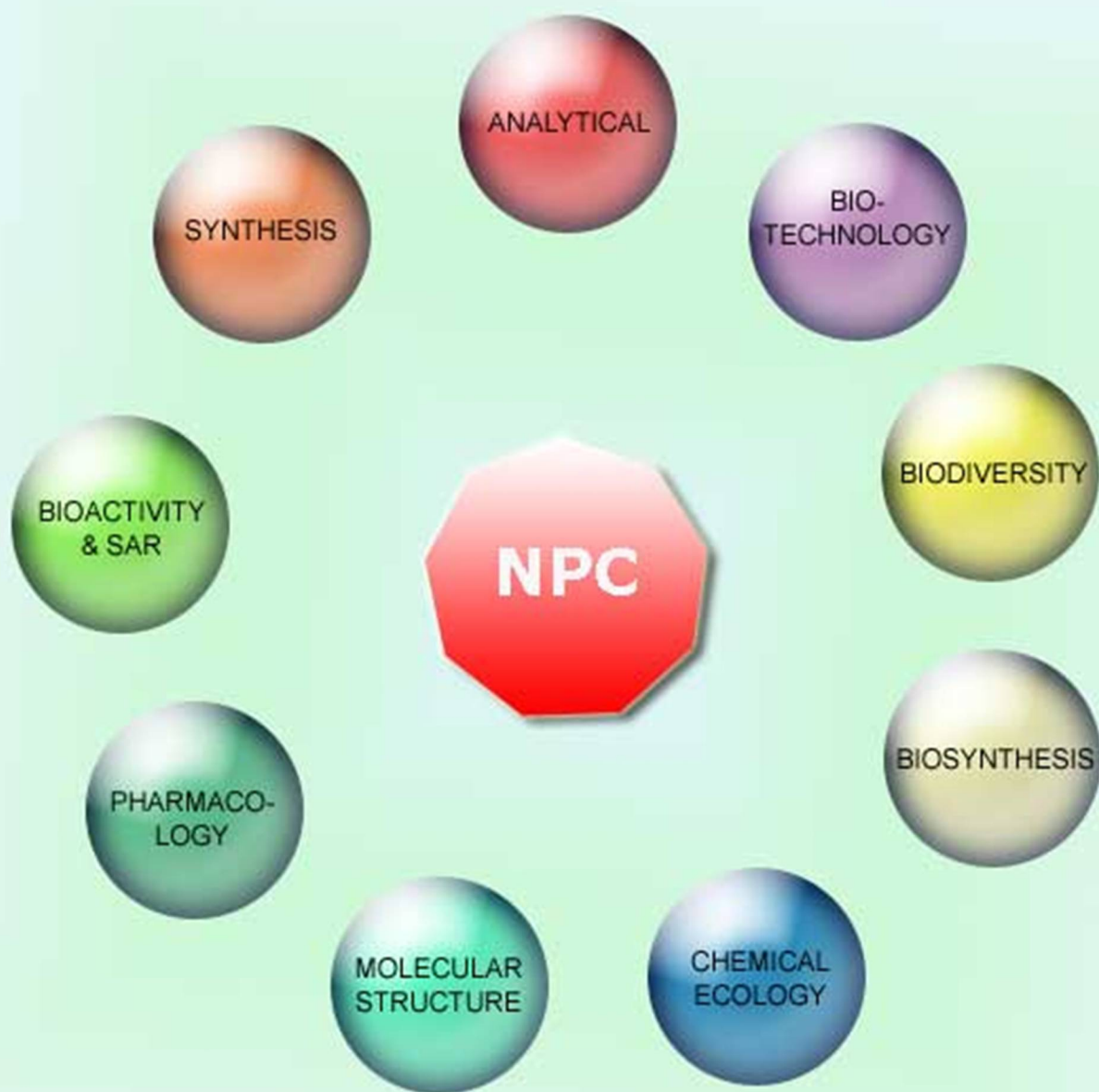
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A Novel Flavonoid and Furoquinoline Alkaloids from *Vepris glomerata* and their Antioxidant Activity

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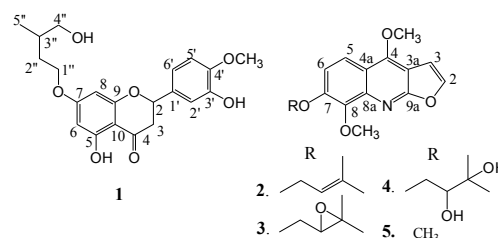
The dichloromethane extract of the aerial part of the plant *Vepris glomerata* (Rutaceae) yielded a new flavonoid, which was accorded the trivial name veprisinol (**1**), together with four known furoquinoline alkaloids: haplopine-3,3'-dimethylallyl ether (**2**), anhydroevoxine (**3**), evoxine (**4**) and skimmianine (**5**). The structures of the compounds were established by 1D and 2D NMR spectroscopy, as well as HREIMS. Compounds **1** and **2** have strong antioxidant potential, similar to and in some instances better than ascorbic acid and can be used as beneficial additives to antioxidant supplements.

Keywords: *Vepris glomerata*, veprisinol, furoquinoline alkaloids, antioxidant activity.

The African *Vepris* species have proved to be a good source of furoquinoline and acridone alkaloids that typify the genus as a whole. *V. bilocularis* has been found to have both furoquinoline as well as acridone alkaloids [1,2], while furoquinoline alkaloids alone have been found in *V. ampody* [3], *V. heterophylla* [4], *V. punctata* [5] and *V. stolzii* [6], and acridone alkaloids alone in *V. fitoravina* and *V. macrophylla* [7]. The alkaloids are reported to possess broad spectrum antimicrobial [8], antiradical [9], antioxidant [10], antiplasmodial [11], anticancer [12] and antimutagenic [13] activities. *V. glomerata* is used in African traditional medicine, where its aqueous root extract is used to treat malaria, epilepsy, psychosis and stroke, when mixed with tea [14]. Earlier pharmacological studies on this plant reported antiplasmodial activities of the ethanol extract [15].

Since the species of Rutaceae are often cited as antimalarials or febrifuges in African traditional medicine [14], and the antioxidant activity of alkaloids [10] and flavonoids [16] has previously been demonstrated, all the five compounds isolated were assessed for antioxidant activity using three methods.

Here we report on the isolation and structure elucidation of a new flavonoid, in addition to four known furoquinoline alkaloids: haplopine-3,3'-dimethylallyl ether (**2**), anhydroevoxine (**3**), evoxine (**4**) and skimmianine (**5**) from the dichloromethane extract of *V. glomerata*, together with their antioxidant activities *in vitro*. The structures of the known compounds **2-5** were determined by comparison



of their physical and spectroscopic data with those reported in literature; **2** and **3** [17], **4** [18] and **5** [19]. Only skimmianine was previously reported from the leaves of *V. glomerata* endemic to Ethiopia, in addition to kokusaginine [20]. It is not apparent if the different compounds found in this study are as a result of either geographical or seasonal differences.

Compound **1** was obtained as a yellow solid. Its mass was established to be 388.1573 amu, based on HREIMS data, corresponding to a molecular formula of $C_{21}H_{24}O_7$, which indicates a double bond equivalence of 10, eight being due to the aromatic rings, one being due to the carbonyl group and one to ring C of the flavanone skeleton. The IR spectrum showed a carbonyl stretching band at 1705 cm^{-1} and a hydroxyl absorption band at 3364 cm^{-1} . This compound was identified as a flavanone based on its characteristic ^1H NMR spectral pattern. The characteristic ABX coupling system of H-2 β , H-3 α and H-3 β appeared at δ_{H} 5.29 (1H, dd, $J = 12.84, 2.84\text{ Hz}$, H-2 β), δ_{H} 3.04 (1H, dd, $J = 17.12, 2.84\text{ Hz}$, H-3 α) and δ_{H} 2.75 (1H, dd, $J = 17.12, 12.84\text{ Hz}$, H-3 β). These signals also showed COSY and NOESY correlations with each other.

Another characteristic pattern was that of the trisubstituted aromatic B ring. The proton resonances of this ring occurred as a singlet at δ_H 7.00 (s, H-2') and doublets at δ_H 6.89 and 6.84 (1H each, d, $J = 8.48$ Hz, H-5' and H-6'). The small coupling constant of about 2 Hz for $J_{H2',H6'}$ could not be detected for the H-2' resonance. The 1H NMR spectrum also showed the presence of a methoxy group at δ_H 3.88 (s), its position at C-4' being confirmed by both a 1D NOE and a NOESY correlation with the resonances at δ_H 6.89 and 6.84 (H-5' and H-6'). Five aromatic C-O resonances were seen at δ_C 164.0, 167.2, 162.8, 145.0 and 147.0 attributed to oxygenation at C-5, 7, 9, 3' and 4'.

A pair of doublets at δ_H 6.02 (1H, d, $J = 1.76$ Hz, H-6) and δ_H 6.00 (1H, d, $J = 1.76$ Hz, H-8) were attributed to the *meta* coupled, H-6 and H-8 protons on ring A. These two proton resonances showed NOESY correlations to 2H-1'' at δ_H 4.02, confirming the position of the side chain at C-7. Its corresponding carbon resonance showed HMBC correlations to two multiplets at δ_H 1.87 (overlapping resonances of H-2''a and H-3'') and δ_H 1.61 (H-2''b). The H-2'' resonances were diastereotopic and appeared as two separate resonances. COSY correlations were also observed between H-1'' and H-2''a and H-2''b and between H-2''b and H-3''. The H-3'' methine proton was coupled to the methyl proton resonance at δ_H 0.95 (d, $J = 6.52$ Hz) attributed to 3H-5'' and the methylene proton at δ_H 3.50 (2H-4'') in the COSY spectrum. These correlations formed a side chain which was attached to ring A by an ether linkage at C-7. Compound **1** was thus identified as 4H-1-benzopyran-4-one, 2, 3-dihydro-5-hydroxy-2-(4'-methoxy-3'-hydroxybenzyl)-7-O-(2-methyl butanol) ether, and given the trivial name veprisinol.

The results of the reducing potential (transformation of Fe^{3+} - Fe^{2+}) of the standard (ascorbic acid) and compounds **1-5** are shown in Figure 1. The activity of haplopine-3,3'-dimethylallyl ether, **2** and veprisinol (**1**) was significantly higher than the activity of the other three alkaloids at all concentrations. However, the reducing power of compound **1** was significantly lower than that of compound **2**. The reducing power of the compounds and standard followed the order: ascorbic acid > **2** > **1** > **3** > **4** > **5**.

The DPPH radical scavenging assay results are shown in Fig. 2. The results revealed that the scavenging activity of the standard ascorbic acid was significantly higher than all other compounds tested. At concentrations of 62.5 $\mu g\ mL^{-1}$ and above, the activity decreased in the order ascorbic acid > **1** > **2** > **4** > **3**, whereas at the lower concentrations, 31.25 and 15.625 $\mu g\ mL^{-1}$, evoxine (**4**) had the highest percentage antioxidant activity of 41%. The activity of compounds **1** and **2** was increased with their concentration and significantly higher than other compounds, particularly at higher concentrations (Figure 2).

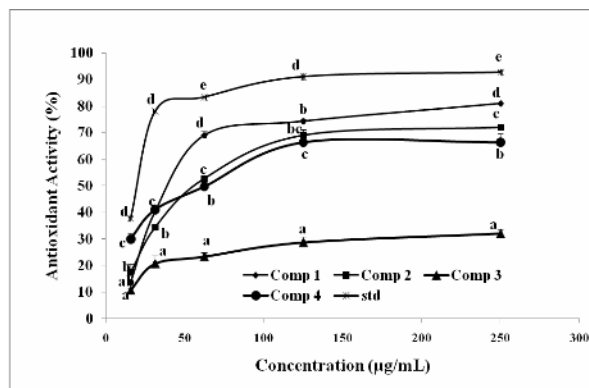


Figure 1: Free radical reducing potential of compounds **1-5** and standard ascorbic acid as evaluated by the spectrophotometric detection of the Fe^{3+} - Fe^{2+} transformation (FRAP method).

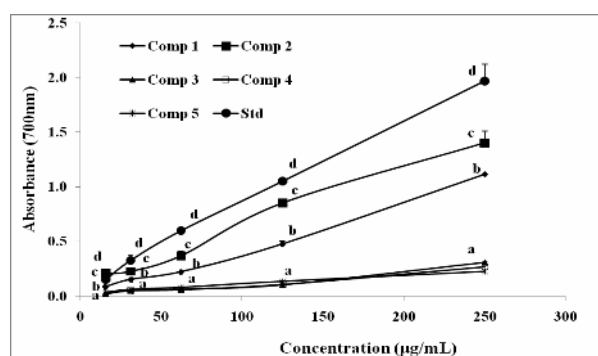


Figure 2: Antioxidant activity of compounds **1-4** and ascorbic acid standard, as measured by the DPPH method.

The hydroxyl radical scavenging activities in the deoxyribose assay are shown in Figure 3. The results revealed that compound **1** possessed significantly higher activity than all the other compounds tested, including the standard, ascorbic acid, at most concentrations. Compounds **1**, **2** and **4** had hydroxyl radical scavenging activity comparable with and in the case of **1** and **2**, better than that of ascorbic acid. Skimmianine (**5**) was not tested in either the DPPH or deoxyribose assays due to insufficient amount.

The three assays revealed that compounds **1** and **2** are good antioxidant compounds, while compound **4** shows high activity at a lower concentration in the DPPH assay. Flavonoids are known to be potent antioxidants and their activity is dependent on their molecular structure. The activity of **1** could be attributed to the hydroxyl (OH) groups in the molecule, which donate hydrogen to reduce the DPPH radical to DPPH-H. The alkaloids **2-5** have the same basic skeleton, the only difference being in their side chain. The reductive ability of **2** may be attributed to the double bond of the isoprenyl unit, rich in delocalized pi-electrons, which are easily donated during reduction of Fe^{3+} to Fe^{2+} . Sang *et al.* also reported that the double bond of the isoprenyl group was responsible for the antioxidant activity of garcinol [21]. The antioxidant activity of **2** in the DPPH assay, like **1**, could also be attributed to the hydroxyl groups in the molecule.

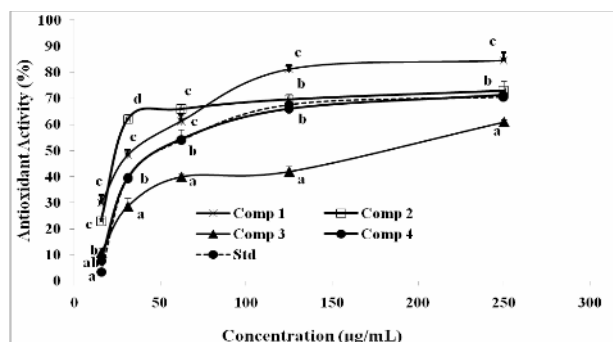


Figure 3: Hydroxyl radical scavenging activity of compounds 1-4 and standard ascorbic acid as measured by the deoxyribose method.

In conclusion, five compounds were isolated (a flavonoid and four alkaloids) from the aerial parts of *V. glomerata*. Verification of their antioxidant activities, as well as comparison with known antioxidants, will provide herbalists and traditional healers with scientific evidence for the use of the aerial parts of this plant as natural antioxidants.

Experimental

General experiment procedures: The melting points were recorded on an Ernst Leitz Wetzlar micro-hot stage melting point apparatus and are uncorrected. UV spectra were obtained on a Varian Cary UV-VIS Spectrophotometer in chloroform. IR spectra were recorded on a Perkin-Elmer Universal ATR Spectrometer. The 1D and 2D NMR spectra were recorded using a Bruker Avance^{III} 400 MHz NMR spectrometer. All the spectra were recorded at room temperature using deuterated chloroform (CDCl₃) as solvent. The HREIMS was measured on a Bruker Micro TOF-QII instrument. Specific rotations were measured at room temperature in chloroform on a PerkinElmerTM, Model 341 Polarimeter with a 10 mm flow tube. The separation, isolation and purification of compounds were carried out by gravity CC and monitored by TLC. Merck silica gel 60 (0.040-0.063 mm) was used for CC. Merck 20 × 20 cm silica gel 60 F₂₅₄ aluminum sheets were used for TLC. TLC plates were analyzed under UV light (254 and 366 nm) before being sprayed with anisaldehyde: concentrated sulfuric acid: methanol [1:2:97] spray reagent and then heated.

Plant material: *Vepris glomerata* was collected from the Rift Valley province of Kenya and identified by Dr S. T. Kariuki from the Department of Botany, Egerton University, Kenya. A voucher specimen (Kiplimo 01) was deposited at the University of KwaZulu-Natal Ward Herbarium, Westville Campus, Durban, South Africa.

Extraction and isolation: The air-dried aerial parts (980 g) of *V. glomerata* were sequentially extracted with *n*-hexane, followed by dichloromethane in a Soxhlet apparatus for 48 h, yielding crude extracts of 46 and 32 g, respectively. The oily residue of the dichloromethane extract obtained after evaporation under vacuum, was separated by CC on silica

gel with *n*-hexane and then increasing the concentration of ethyl acetate from 10 to 80% in *n*-hexane, to give 10 fractions (fr.); fr. 8-16 (1.27 g), fr. 17-19 (0.5 g), fr. 20-26 (2.36 g), fr. 27-32 (2.35 g), fr. 33-39 (1 g), fr. 40-43 (2.1 g), fr. 44-49 (0.5 g), fr. 52-56 (3.9 g), fr. 57-62 (1.75 g) and fr. 63-67 (5.1 g).

Fraction 52-56 was separated by CC with *n*-hexane/EtOAc (7:3) as the solvent to afford sub-fractions A-C. Sub-fraction A was further purified using 100% dichloromethane to afford compound 2, a green solid (51 mg). Sub-fraction B yielded compound 3, a brownish solid (43 mg), which needed no further purification. Sub-fraction C was crystallized in methanol to afford 4 (62 mg). Fraction 44-49 was purified using 100% dichloromethane to afford 5 (60 mg). Fraction 63-67 was separated with *n*-hexane/EtOAc (4:1) to yield 4 sub-fractions A-D. Sub-fraction B was crystallized in methanol to afford yellow crystals of compound 1 (18 mg).

Veprisinol (1)

4H-1-Benzopyran-4-one, 2, 3-dihydro-5-hydroxy-2-(4'-methoxy-3'-hydroxybenzyl)-7-O-(2-methyl butanol) ether

Yellow solid.

M.p: 78-80°C.

[α]_D²⁰: +55.30 (c 0.056, CHCl₃).

IR: 3364 (O-H), 2928, 1705 (C=O), 1636, 1512, 1162 cm⁻¹. UV λ_{max} (CHCl₃) nm (log ε): 337 (4.45), 285 (5.13), 239 (5.44).

¹H NMR (400 MHz, CDCl₃): 11.97 (H, s, OH), 7.00 (H, s, H-2''), 6.89 (H, d, *J* = 8.28 Hz, H-5'), 6.84 (H, d, *J* = 8.28 Hz, H-6'), 6.02 (H, d, *J* = 1.76 Hz, H-6), 6.00 (H, d, *J* = 1.76 Hz, H-8), 5.29 (H, dd, *J* = 12.84, 2.84 Hz, H-2β), 4.02 (2H, dd, *J* = 12.88, 6.24 Hz, 2H-1''), 3.88 (3H, s, OCH₃), 3.50 (2H, d, *J* = 5.68 Hz, 2H-4''), 3.04 (H, dd, *J* = 17.12, 12.84 Hz, H-3α), 2.75 (H, dd, *J* = 17.12, 2.84 Hz, H-3β), 1.87 (2H, m, H-2''a and H-3''), 1.61 (H, m, H-2''b), 0.95 (3H, d, *J* = 6.52 Hz, H-5'').

¹³C NMR: 195.97 (C, C-4), 167.29 (C, C-7), 164.05 (C, C-5), 162.85 (C, C-9), 147.02 (C, C-4'), 145.93 (C, C-3'), 131.52 (C, C-1'), 118.15 (CH, C-5'), 112.71 (CH, C-2'), 110.71 (CH, C-6'), 103.11 (C, C-10), 95.54 (CH, C-6), 94.60 (CH, C-8), 78.92 (CH, C-2), 67.86 (CH₂, C-4''), 66.70 (CH₂, C-1''), 56.06 (OCH₃), 43.15 (CH₂, C-3), 32.94 (CH, C-3''), 32.37 (CH₂, C-2''), 16.60 (CH₃, C-5'').

HREIMS *m/z* 388.1573 [M]⁺ (calcd. for C₂₁H₂₄O₇, 388.1522)

Antioxidant activity: The total reducing power was determined according to the method described previously [22]. The free radical scavenging activity (antioxidant capacity) of the plant phytochemicals on the stable radical 2, 2-diphenyl-β-picrylhydrazyl (DPPH) was evaluated by the method established by Shirwaikar *et al.* [23], and the deoxyribose assay for hydroxyl radical scavenging activity was performed as described previously by Chung *et al.* [24].

Statistical analysis: The data in Figures 1-3 are presented as mean $\pm$ SD of triplicates. ^{a-d}Values with different superscript letters for a given concentration are significantly different from each of the other compounds. The data were statistically analyzed using a statistical software program SPSS (SPSS for Windows, version 18, SPSS Science, Chicago, IL, USA). One-way analysis of

variance (ANOVA) followed by Tukey's multiple range post-hoc test was employed to find the differences. The data were considered significantly different at $p < 0.05$.

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Ferric Reducing, Antiradical and β-Carotene Bleaching Activities of Nicotinic Acid and Picolinic Acid Bioconjugates of Curcumin	
Archana Pandey, Kanti Bhooshan Pandey, Ravindra Kumar Gupta and Syed Ibrahim Rizvi	1877
Antiviral Activities of Diarylheptanoids Isolated from <i>Alpinia officinarum</i> against Respiratory Syncytial Virus, Poliovirus, Measles Virus, and Herpes Simplex Virus Type 1 <i>in vitro</i>	
Katsuhiko Konno, Rie Sawamura, Yi Sun, Ken Yasukawa, Tomomi Shimizu, Wataru Watanabe, Masahiko Kato, Ryuichi Yamamoto and Masahiko Kurokawa	1881
Isolation of C-glycosyl Xanthenes from <i>Coffea pseudozanguebariae</i> and Their Location	
Pascale Talamond, Geneviève Conejero, Jean-Luc Verdeil and Jean-Luc Poëssel	1885
Antifungal Activity and Isomerization of Octadecyl <i>p</i>-coumarates from <i>Ipomoea carnea</i> subsp. <i>fistulosa</i>	
Eugene Sebastian J. Nidiry, Girija Ganeshan and Ankanahalli N. Lokesh	1889
New Glucose Esters from the Fresh Leaves of <i>Jacaranda mimosaeifolia</i>	
Christianah A. Elusiyan and Tiwalade A. Olugbade	1893
Shamiminol: A New Aromatic Glycoside from the Stem Bark of <i>Bombax ceiba</i>	
Shaheen Faizi, Sadia Zikr-Ur-Rehman and Muhammad Ali Versiani	1897
Two New Phenolic Glycosides from <i>Viburnum plicatum</i> var. <i>plicatum</i> f. <i>plicatum</i>	
Saki Katagiri, Yoshiki Watanabe, Yasunori Yaoita, Masao Kikuchi and Koichi Machida	1901
Antimicrobial Chemical Constituents from the Endophytic Fungus <i>Phomopsis</i> sp. from <i>Notobasis syriaca</i>	
Hidayat Hussain, Michel Kenne Tchimine, Ishtiaq Ahmed, Kathrin Meier, Michael Steinert, Siegfried Draeger, Barbara Schulz and Karsten Krohn	1905
Phomosines H–J, Novel Highly Substituted Biaryl Ethers, Isolated from the Endophytic Fungus <i>Phomopsis</i> sp. from <i>Ligustrum vulgare</i>	
Karsten Krohn, Umar Farooq, Hidayat Hussain, Ishtiaq Ahmed, Joachim Rheinheimer, Siegfried Draeger, Barbara Schulz and Teunis van Ree	1907
Isolation and Characterization of a new Benzofuran from the Fungus <i>Alternaria</i> sp. (HS-3) Associated with a Sea Cucumber	
Xuekui Xia, Jun Qi, Fang Wei, Airong Jia, Wenpeng Yuan, Xiumei Meng, Miansong Zhang, Changheng Liu and Changyun Wang	1913
Potent Toxic Macrocyclic Trichothecenes from the Marine-Derived Fungus <i>Myrothecium verrucaria</i> Hmp-F73	
Li Zhao, Li Liu, Nan Wang, Shu-Jin Wang, Jing-Chun Hu and Jin-Ming Gao	1915
Synthesis and Bioactivity of Novel Coumarin Derivatives	
Ai-Ying Guan, Chang-Ling Liu, Miao Li, Zhi-Nian Li, Ming-Xing Zhang and Hong Zhang	1917
Kinase Inhibitory, Haemolytic and Cytotoxic Activity of Three Deep-water Sponges from North Western Australia and their Fatty Acid Composition	
Ana Zivanovic, Natalie J. Pastro, Jane Fromont, Murray Thomson and Danielle Skropeta	1921
Antimicrobial and Cytotoxic Effects of Mexican Medicinal Plants	
Maria del Rosario Jacobo-Salcedo, Angel Josabad Alonso-Castro, Luis A. Salazar-Olivo, Candy Carranza-Alvarez, Luis Ángel González-Espíndola, Fabiola Domínguez, Sandra Patricia Maciel-Torres, Concepción García-Lujan, Marisela del Rocio González-Martínez, Maricela Gómez-Sánchez, Eduardo Estrada-Castillón, Rocio Zapata-Bustos, Pedro Medellín-Milán and Alejandro García-Carrancá	1925
Chemometrics Evaluation of the Herbal Drug <i>Andrographis paniculata</i>	
Shiv Narayan Sharma, Zenu Jha and D. K. Sharma	1929
<i>Garcinia cambogia</i> Leaf and Seawater for Tannase Production by Marine <i>Aspergillus awamori</i> BTMFW032 under Slurry State Fermentation	
Beena P. S, Soorej M. Basheer, Sarita G. Bhat and Chandrasekaran M	1933
Gas Chromatographic Quantitative Analysis of Methanol in Wine: Operative Conditions, Optimization and Calibration Model Choice	
Rosario Caruso, Grazia Laura Gambino, Monica Scordino, Leonardo Sabatino, Pasqualino Traulo and Giacomo Gagliano	1939
Composition and Biological Potential of Essential Oil from <i>Thelechitonina trilobata</i> Growing in South Africa	
Jamie Peebles, Ephraim Gwebu, Opeoluwa Oyediji, Sarah Nanyonga, Nokuthula Kunene, David Jackson, William Setzer and Adebola Oyediji	1945
Chemical Composition and Antibacterial Activity of Essential oil from <i>Salvia mukerjeei</i>	
Lalit Mohan, Anuradha Negi, Anand B. Melkani and Vasu Dev	1949
<u>Review/Account</u>	
Revealing Indigenous Indonesian Traditional Medicine: Anti-infective Agents	
Ari S. Nugraha and Paul A. Keller	1953

Natural Product Communications

2011

Volume 6, Number 12

Contents

<u>Original Paper</u>	<u>Page</u>
Sibiralactone: A New Monoterpene from <i>Sibiraea angustata</i> Guangbo Xie, Xianlong Wang, Tibor Kurtán, Attila Mándi and Tianzhi Wang	1799
Bioconversion of Proposed Precursors into Theobroxide and Related Compounds Peng Li, Kosaku Takahashi, Ahmed Elkhateeb, Hideyuki Matsuura, Teruhiko Yoshihara and Kensuke Nabeta	1801
Microbial Hydroxylation of <i>S</i>-(-)-Perillyl Alcohol by <i>Fusarium heterosporium</i> Ismail Kiran	1805
A Phytochemical Investigation of <i>Zanthoxylum setulosum</i> Tameka M. Walker, Bernhard Vogler, Debra M. Moriarity, William A. Haber and William N. Setzer	1807
Cytotoxic Cembranoids from the Red Sea Soft Coral <i>Sarcophyton glaucum</i> Mohamed-Elamir F. Hegazy, Ahmed A. El-Beih, Alaa Y. Moustafa, Abdelhamed A. Hamdy, Montaser A. Alhammad, Rehab M. Selim, Mohamed Abdel-Rehim and Paul W. Paré	1809
C-Lactam Derivatives of Oleonic Acid. The synthesis of C-lactam by Beckmann rearrangement of C-oxime Barbara Bednarczyk – Cwynar	1813
Analysis of Native Carotenoid Composition of Sweet Bell Peppers by Serially Coupled C₃₀ Columns Daniele Giuffrida, Paola Dugo, Giacomo Dugo, Germana Torre and Luigi Mondello	1817
New Antifungal Cholestane and Aldehyde Derivatives from the Red Alga <i>Laurencia papillosa</i> Walied M. Alarif, Sultan S. Al-Lihaibi, Ahmed Abdel-Lateff and Seif-Eldin N. Ayyad	1821
Steroidal Saponins from the Fruits of <i>Cestrum ruizteranianum</i> Elier Galarraga M., Anne-Claire Mitaine-Offer, Juan Manuel Amaro-Luis, Tomofumi Miyamoto, Chiaki Tanaka, Laurent Pouységu, Stéphane Quideau, Luis B. Rojas and Marie-Aleth Lacaille-Dubois	1825
Isolation and Cholinesterase Activity of Amaryllidaceae Alkaloids from <i>Nerine bowdenii</i> Lucie Cahliková, Stanislav Zavadil, Kateřina Macáková, Irena Valterová, Andrea Kulhánková, Anna Hošťálková, Jiří Kuneš and Lubomír Opletal	1827
HPLC Determination of Majdine in <i>Vinca herbacea</i> Natia Gagua, Beatrice Baghdikian, Fathi Mabrouki, Riad Elias, Valentina Vachnadze, Aliosha Bakuridze and Evelyne Ollivier	1831
Pyridine Metabolism and Trigonelline Synthesis in Leaves of the Mangrove Legume trees <i>Derris indica</i> (<i>Millettia pinnata</i>) and <i>Caesalpinia crista</i> Yuling Yin, Hamako Sasamoto and Hiroshi Ashihara	1835
Anti-adipogenic Activity of <i>Cordyceps militaris</i> in 3T3-L1 Cells Qing Liu, In Pyo Hong, Mi-Jeong Ahn, Hwan-Soo Yoo, Sang-Bae Han, Bang Yeon Hwang and Mi Kyeong Lee	1839
Two New Cyclopeptides and One New Nonenolide from <i>Xylaria</i> sp. 101 Yao-Yao Li, Zhi-Yu Hu, and Yue-Mao Shen	1843
A Novel Flavonoid and Furoquinoline Alkaloids from <i>Vepris glomerata</i> and their Antioxidant Activity Joyce J. Kiplimo, Md. Shahidul Islam and Neil A. Koobanally	1847
Flavonoid Constituents and Free Radical Scavenging Activity of <i>Alchemilla mollis</i> Antoaneta Trendafilova, Milka Todorova, Milena Nikolova, Anna Gavrilova and Antonina Vitkova	1851
Ultrasound-assisted Extraction of Total Phenols and Flavonoids from Dry Tobacco (<i>Nicotiana tabacum</i>) Leaves Ivana T. Karabegović, Vlada B. Veljković and Miodrag L. Lazić	1855
Characterization of Polyphenolic Compounds in Unripe Chinotto (<i>Citrus myrtifolia</i>) Fruit by HPLC/PDA/ESI/MS-MS Monica Scordino, Leonardo Sabatino, Adalgisa Belligno and Giacomo Gagliano	1857
Bioactive Compounds, RP-HPLC Analysis of Phenolics, and Antioxidant Activity of Some Portuguese Shrub Species Extracts Ángelo Luís, Fernanda Domingues and Ana Paula Duarte	1863
HPLC/PDA/ESI-MS Evaluation of Saffron (<i>Crocus sativus</i> L.) Adulteration Leonardo Sabatino, Monica Scordino, Maria Gargano, Adalgisa Belligno, Pasqualino Traulo and Giacomo Gagliano	1873

Continued inside backcover