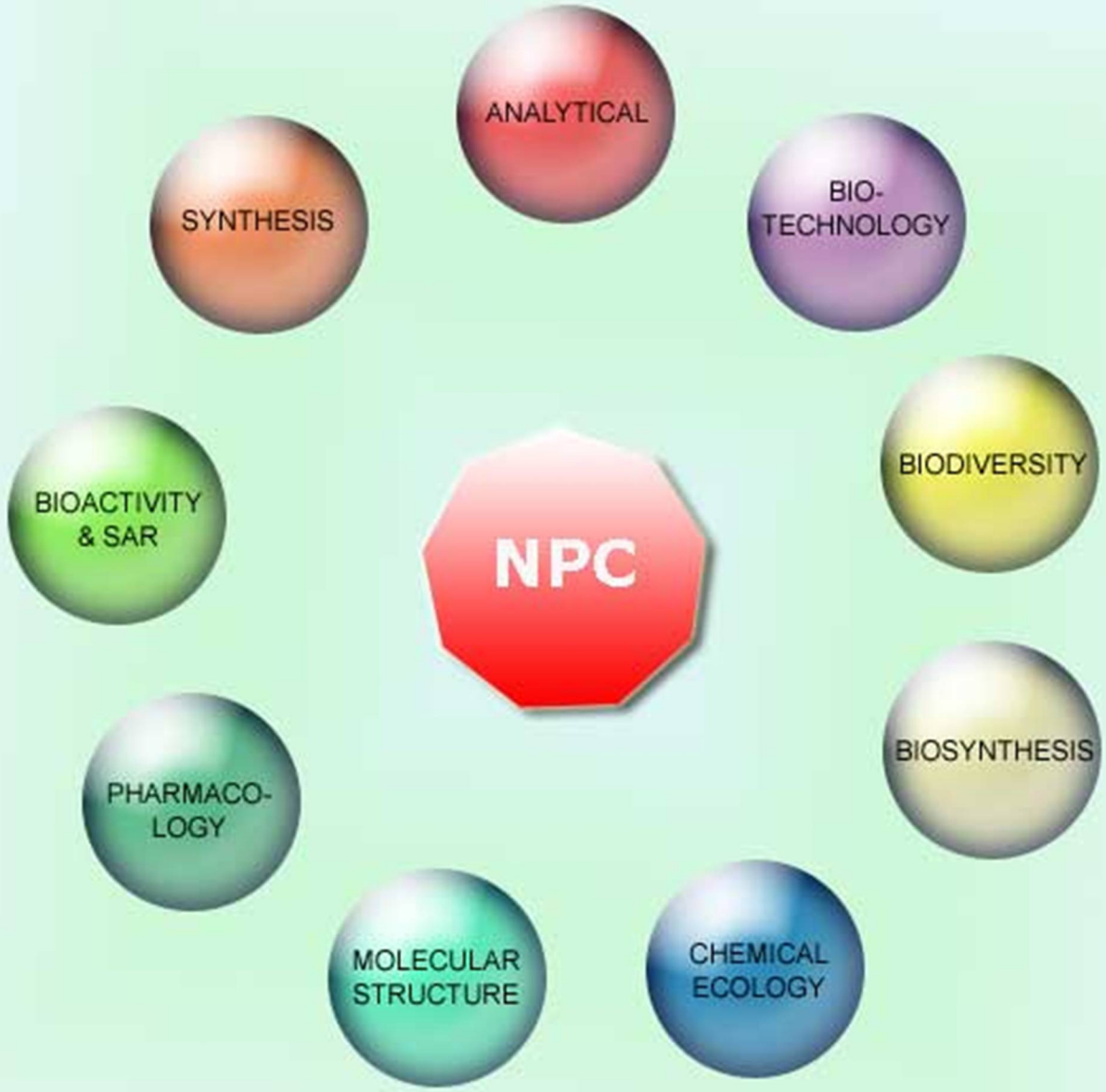
NATURAL PRODUCT COMMUNICATIONS

An International Journal for Communications and Reviews Covering all Aspects of Natural Products Research



Volume 6. Issue 12. Pages 1799-1968. 2011
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

NPC Natural Product Communications

EDITOR-IN-CHIEF

DR. PAWAN K AGRAWAL
Natural Product Inc.
7963, Anderson Park Lane,
Westerville, Ohio 43081, USA
agrawal@naturalproduct.us

HONORARY EDITOR

PROFESSOR GERALD BLUNDEN
The School of Pharmacy & Biomedical Sciences,
University of Portsmouth,
Portsmouth, PO1 2DT U.K.
axuf64@dsl.pipex.com

EDITORS

PROFESSOR ALESSANDRA BRACA
Dipartimento di Chimica Bioorganicae Biofarmacia,
Universita di Pisa,
via Bonanno 33, 56126 Pisa, Italy
braca@farm.unipi.it

PROFESSOR DEAN GUO
State Key Laboratory of Natural and Biomimetic Drugs,
School of Pharmaceutical Sciences,
Peking University,
Beijing 100083, China
gda5958@163.com

PROFESSOR YOSHIHIRO MIMAKI
School of Pharmacy,
Tokyo University of Pharmacy and Life Sciences,
Horinouchi 1432-1, Hachioji, Tokyo 192-0392, Japan
mimakiy@ps.toyaku.ac.jp

PROFESSOR STEPHEN G. PYNE
Department of Chemistry
University of Wollongong
Wollongong, New South Wales, 2522, Australia
spyne@uow.edu.au

PROFESSOR MANFRED G. REINECKE
Department of Chemistry,
Texas Christian University,
Forts Worth, TX 76129, USA
m.reinecke@tcu.edu

PROFESSOR WILLIAM N. SETZER
Department of Chemistry
The University of Alabama in Huntsville
Huntsville, AL 35809, USA
wsetzer@chemistry.uah.edu

PROFESSOR YASUHIRO TEZUKA
Institute of Natural Medicine
Institute of Natural Medicine, University of Toyama,
2630-Sugitani, Toyama 930-0194, Japan
tezuka@inm.u-toyama.ac.jp

PROFESSOR DAVID E. THURSTON
Department of Pharmaceutical and Biological Chemistry,
The School of Pharmacy,
University of London, 29-39 Brunswick Square,
London WC1N 1AX, UK
david.thurston@pharmacy.ac.uk

ADVISORY BOARD

Prof. Berhanu M. Abegaz
Gaborone, Botswana

Prof. Viqar Uddin Ahmad
Karachi, Pakistan

Prof. Øyvind M. Andersen
Bergen, Norway

Prof. Giovanni Appendino
Novara, Italy

Prof. Yoshinori Asakawa
Tokushima, Japan

Prof. Lee Banting
Portsmouth, U.K.

Prof. Julie Banerji
Kolkata, India

Prof. Alejandro F. Barrero
Granada, Spain

Prof. Anna R. Bilia
Florence, Italy

Prof. Maurizio Bruno
Palermo, Italy

Prof. César A. N. Catalán
Tucumán,Argentina

Prof. Josep Coll
Barcelona, Spain

Prof. Geoffrey Cordell
Chicago, IL, USA

Prof. Cristina Gracia-Viguera
Murcia, Spain

Prof. Duvvuru Gunasekar
Tirupati, India

Prof. A.A. Leslie Gunatilaka
Tucson, AZ, USA

Prof. Kurt Hostettmann
Lausanne, Switzerland

Prof. Martin A. Iglesias Arteaga
Mexico, D. F, Mexico

Prof. Jerzy Jaroszewski
Copenhagen, Denmark

Prof. Leopold Jirovetz
Vienna, Austria

Prof. Karsten Krohn
Paderborn, Germany

Prof. Hartmut Laatsch
Gottingen, Germany

Prof. Marie Lacaille-Dubois
Dijon, France

Prof. Shoei-Sheng Lee
Taipei, Taiwan

Prof. Francisco Macias
Cadiz, Spain

Prof. Imre Mathe
Szeged, Hungary

Prof. Joseph Michael
Johannesburg, South Africa

Prof. Ermino Murano
Trieste, Italy

Prof. M. Soledade C. Pedras
Saskatoon, Canada

Prof. Luc Pieters
Antwerp, Belgium

Prof. Peter Proksch
Düsseldorf, Germany

Prof. Phila Raharivelomanana
Tahiti, French Polynesia

Prof. Monique Simmonds
Richmond, UK

Prof. Valentin Stonik
Vladivostok, Russia

Prof. Winston F. Tinto
Barbados, West Indies

Prof. Karen Valant-Vetschera
Vienna, Austria

Prof. Peter G. Waterman
Lismore, Australia

INFORMATION FOR AUTHORS

Full details of how to submit a manuscript for publication in Natural Product Communications are given in Information for Authors on our Web site http://www.naturalproduct.us.

Authors may reproduce/republish portions of their published contribution without seeking permission from NPC, provided that any such republication is accompanied by an acknowledgment (original citation)-Reproduced by permission of Natural Product Communications. Any unauthorized reproduction, transmission or storage may result in either civil or criminal liability.

The publication of each of the articles contained herein is protected by copyright. Except as allowed under national "fair use" laws, copying is not permitted by any means or for any purpose, such as for distribution to any third party (whether by sale, loan, gift, or otherwise); as agent (express or implied) of any third party; for purposes of advertising or promotion; or to create collective or derivative works. Such permission requests, or other inquiries, should be addressed to the Natural Product Inc. (NPI). A photocopy license is available from the NPI for institutional subscribers that need to make multiple copies of single articles for internal study or research purposes.

To Subscribe: Natural Product Communications is a journal published monthly. 2011 subscription price: US$1,995 (Print, ISSN# 1934-578X); US$1,995 (Web edition, ISSN# 1555-9475); US$2,495 (Print + single site online); US$595 (Personal online). Orders should be addressed to Subscription Department, Natural Product Communications, Natural Product Inc., 7963 Anderson Park Lane, Westerville, Ohio 43081, USA. Subscriptions are renewed on an annual basis. Claims for nonreceipt of issues will be honored if made within three months of publication of the issue. All issues are dispatched by airmail throughout the world, excluding the USA and Canada.

NPC Natural Product Communications

2011
Vol. 6
No. 12
1851 - 1854

Flavonoid Constituents and Free Radical Scavenging Activity of *Alchemilla mollis*

Antoaneta Trendafilova[a,*], Milka Todorova[a], Milena Nikolova[b], Anna Gavrilova[b] and Antonina Vitkova[b]

[a]*Institute of Organic Chemistry with Centre of Phytochemistry*, *Bulgarian Academy of Sciences*, *Acad. G. Bonchev Str.*, *bl. 9*, *1113 Sofia*, *Bulgaria*

[b]*Institute of Biodiversity and Ecosystem Research*, *Bulgarian Academy of Sciences*, *2 Gagarin Str.*, *1113 Sofia*, *Bulgaria*

trendaf@orgchm.bas.bg

Received: June 29th, 2011; Accepted: October 24th, 2011

Antioxidant capacity of the methanolic extract of *Alchemilla mollis* was measured by its ability to scavenge the DPPH radical. The EtOAc fraction obtained after partition of the total extract was found to be the most active radical scavenger (IC_{50} 9.8 ± 1.8 μg/mL) and was subjected to fractionation by Sephadex LH-20 CC. Further purification by RP-18 CC led to the isolation of eight flavonoid glycosides: *cis*- and *trans*-tiliroside (**1** and **2**), rhodiolgin (**3**), hyperoside (**4**), isoquercitrin (**5**), miquelianin (**6**), sinocrassoside D_2 (**7**), and gossypetin-3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranoside (**8**). It was found that **8** is a new compound and its antioxidant activity is also reported. Identification of the isolated compounds was carried out by spectroscopic and spectrometric analysis (1D and 2D NMR, UV and MS).

Keywords: *Alchemilla mollis,* Rosaceae, flavonoid glycosides, antioxidant activity.

Alchemilla mollis (Buser) Rothm. (Rosaceae) is a herbaceous perennial plant that grows in mountainous regions of the Balkan Peninsula (Romania, Greece and Bulgaria) [1]. The species, with its only known locality in the National Park "Central Balkan", is critically endangered in Bulgaria according to the IUCN criteria and was included in the Red Book of Bulgaria, the Red List of Bulgarian vascular plants, the Biological Diversity Act, and the Medicinal Plants Act [2a-2c]. Because of its very limited occurrence, its cultivation has been started recently [3,4].

A. mollis forms part of the commercial drug "Herba Alchemillae", an aggregate of species collectively referred to as Lady's mantle. The drug possesses astringent, diuretic and antispasmodic properties, and is commonly used in traditional medicine as a cure for excessive menstruation and wounds [5,6]. Different studies showed that the phenolic compounds (for example, tannins, and flavonoids) present in the plant are responsible for the pharmacological activity of Lady's mantle [7a-7d]. Surprisingly, the literature data concerning the chemical composition of *A. mollis* have proved to be quite limited, with only a few reports on total flavonoid and tannin contents of the plant [8a-8c]. Consequently, the aim of the present work was to study the flavonoid constituents of this species and their antioxidant capacity.

The DPPH scavenging assay is widely used for preliminary evaluation of the antioxidant potential of extracts and individual compounds [10,11]. In this work, the MeOH extract of *A. mollis* and its fractions obtained after re-extraction with light petroleum (PE), chloroform ($CHCl_3$), and ethyl acetate (EtOAc), as well as the remaining H_2O residue were studied for their general antioxidant effect, as indicated by their potential to scavenge the stable DPPH radical.

Table 1: DPPH radical scavenging activity of *A. mollis* total methanolic extract, fractions and individual compounds.

	IC_{50} (μg/mL)
total MeOH extract	31.7 ± 4.9
PE fraction	>200
$CHCl_3$ fraction	>200
EtOAc fraction	9.8 ±1.8
H_2O residue	42.5 ± 4.3
Quercetin (Reference)	3.2 ± 0.4
Hyperoside (**4**)	5.1 ± 0.4
Compound **8**	4.9 ± 0.5

The total MeOH extract exhibited a significant dose dependent inhibition of DPPH activity with 50% inhibition (IC_{50}) at a concentration of 31.7 ± 4.9 μg/mL. As shown in Table 1, the scavenging activities of the fractions on the DPPH radical increased in the order of PE<$CHCl_3$ <H_2O<EtOAc. Although the DPPH free radical scavenging ability of the EtOAc fraction (IC_{50} 9.8 ± 1.8 μg/mL) was less than that of quercetin (IC_{50} 3.2 ± 0.4 μg/mL) it was evident that the EtOAc-soluble fraction had hydrogen donating ability and could serve as either a free radical inhibitor or scavenger, possibly acting as a primary antioxidant.

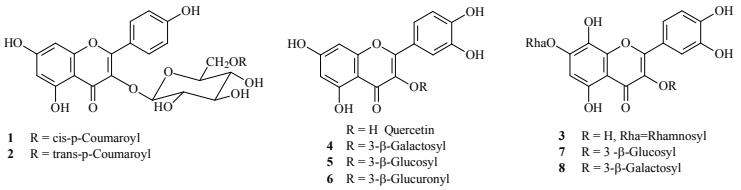


Figure 1: Structures of flavonoid glycosides **1-8**.

The most active EtOAc-soluble fraction was subjected to Sephadex LH-20 column chromatography. Further purification by RP-18 silica gel column chromatography afforded eight flavonoid glycosides. The structures (Figure 1) of the known *cis*- and *trans*-tiliroside (**1** and **2**) [11], rhodiolgin (**3**) [12], hyperoside (**4**) [13], isoquercitrin (**5**) [13], miquelianin (**6**) [13] and sinocrassoside D_2 (**7**) [14] were determined by comparison of their spectral data (UV, ^{1}H NMR, ^{13}C NMR and MS) with those published previously.

Table 2: ^{1}H and ^{13}C NMR spectroscopic data of compound **8** (CD_3OD).

Position	δ_H J (Hz)	δ_C	Position	δ_H J (Hz)	δ_C
2		159.4			
3		135.3	1”	5.20 d (7.2)	105.4
4		180.1	2”	3.85 m*	73.2
5		154.3	3”	3.65 m*	75.1
6	6.67 s	99.9	4”	3.85 m*	70.1
7		152.2	5”	3.50 m*	77.2
8		128.5	6”	3.50 m*	62.0
9		146.2			
10		107.1			
1’		123.0	1’”	5.54 brs	100.9
2’	7.94 d (2.2)	116.1	2’”	4.14 brs	71.7
3’		145.8	3’”	3.99 dd (2.8, 9.4)	72.1
4’		150.2	4’”	3.65 m*	73.8
5’	6.87 d (8.5)	118.1	5’”	3.55 m*	71.3
6’	7.72 dd (2.2,8.5)	123.5	6’”	1.26 d (6.2)	18.1

*overlapped signals

Compound **8**, isolated as yellowish amorphous powder, displayed spectral properties very similar to those of sinocrassoside D_2 (**7**) [14]. The negative HR-ESI-MS of **8** showed a $[M-H]^+$ peak at *m/z* 625.14035, which corresponded to a molecular formula $C_{27}H_{29}O_7$. The ^{1}H NMR spectrum (Table 2) contained signals assignable to a gossypetin moiety [δ_H 6.67 (1H, s, H-6), 6.87 (1H, d, J = 8.5 Hz, H-5’), 7.72 (1H, dd, J = 2.2, 8.5 Hz, H-6’), 7.94 (1H, d, J = 2.2 Hz, H-2’)], an α-L-rhamnopyranosyl unit [δ_H 1.26 (3H, d, J=6.2 Hz) and δ_H 5.54 (1H, brs)] and the doublet at δ_H 5.20 (J = 7.2 Hz) characteristic of an anomeric proton of a sugar moiety with β-configuration. This was identified as D-galactose after acid hydrolysis of **8** and confirmed by co-TLC with an authentic sample. The full NMR assignments and connectivities of **8** were determined by HMBC and NOESY data analysis (Figure 2). Thus, the observed HMBC long-range correlations between anomeric proton H-1” (δ_H 5.20) and C-3 δ_C (135.3), as well as between the second anomeric proton H-1’” (δ_H 5.54) and C-7 (δ_C 152.2) supported the location of sugar moieties at C-3 and C-7. Furthermore, NOE interactions of the aromatic singlet at δ_H 6.67 (H-6) with H-1’” and H-6’” confirmed the position of the

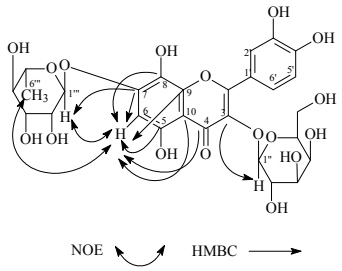


Figure 2: Significant HMBC and NOE correlations in **8**.

α-L-rhamnopyranosyl unit at C-7. Thus, the new compound (**8**) was identified as gossypetin-3-β-D-galactopyranosyl-7-α-L-rhamnopyranoside.

Finally, the antioxidant activity of the new compound **8** was evaluated by the DPPH free radical scavenging assay (Table 1) and compared with that of hyperoside (**4**) and quercetin. As can be seen, **8** exhibited antioxidant activity against the DPPH radical with an IC_{50} value (4.9 ± 0.5 μg/mL) similar to that of hyperoside (IC_{50} 5.1 ± 0.4 μg/mL) and lower than that of quercetin (IC_{50} 3.2 ± 0.4 μg/mL). This significant radical scavenging activity of **8** could be explained by the presence of *ortho*-dihydroxyl groups at C-3’ and C-4’ in ring B of the flavonoid structure [15].

The results described above showed that the EtOAc fraction obtained from the total methanolic extract of *A. mollis* was characterized by the presence of three different types of flavonoid glycosides – kaempferol (**1** and **2**), quercetin (**4**, **5** and **6**) and gossypetin (**3**, **7** and **8**). It is noteworthy, that kaempferol and quercetin glycosides have been previously found in *A. speciosa* [16], *A. xanthochlora* [17,18] and *A. vulgaris* [19], while gossypetin glycosides were detected for the first time in the genus *Alchemilla*. These differences in the chemical composition of the investigated species could be of chemotaxonomic importance.

Experimental

General: UV spectra were taken on a Helios Gamma spectrophotometer (Thermo Electron Corporation). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II+ 600 NMR spectrometer with an operating frequency of 600.13 MHz (^{1}H) and 150.903 MHz (^{13}C), using TMS as internal standard and CD_3OD as solvent. Chemical shifts (δ) are expressed in ppm and coupling constants (J) in Hertz. 1D and 2D NMR spectra were recorded using the standard Bruker pulse sequence. The HR-ESI-TOF-MS

was measured using a 6210 Time-of-flight LC/MS system (Agilent technologies) coupled with a HPLC instrument 1200 Series (Agilent Technologies). TLC analyses were carried out on silica gel 60 F_{254} precoated plates (Merck). The compounds were visualized by spraying with either H_2SO_4 or NP-reagent (Fluka). For open column chromatography, Sephadex LH-20 (Fluka) was used as stationary phase. A LiChroprep RP-18 column (Merck) was used for medium pressure liquid chromatography (MPLC).

Plant material: *A. mollis* plants from a native Bulgarian population (Stara planina mt. -1150 m a.s.l.), cultivated in the experimental field of the Institute of Biodiversity and Ecosystem Research (Sofia – 570 m a.s.l.), were used in the experiments. The aerial flowering parts of the plants were collected within phenophase – full blossoming, air-dried and kept in the dark. The voucher specimen (SOM 159980) was deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia.

Extraction and isolation: Powdered plant material (70 g) was extracted with MeOH (3 x 1 L) at room temperature in an ultrasonic bath for 30 min each time. After filtration, the solvent from the combined extracts was evaporated under vacuum to give the total methanolic extract (MeOH, 9.10 g). This was further dissolved in distilled water (200 mL) and partitioned with light petroleum (PE, 3 x 80 mL), chloroform ($CHCl_3$, 3 x 80 mL) and ethyl acetate (EtOAc, 3 x 80 mL) to yield PE (0.57 g), $CHCl_3$ (0.28 g) and EtOAc (0.44 g) fractions. The remaining aqueous phase was evaporated to dryness (7.51 g).

The EtOAc extract was dissolved in MeOH (15 mL) and filtered through celite in order to remove insoluble parts. The clear methanolic solution was concentrated to 5 mL and applied to a Sephadex LH-20 column (equilibrated with MeOH) to give 4 main fractions [TLC: Silica gel, EtOAc/MeOH/H_2O, 5:0.8:0.6, and EtOAc/HCOOH/ CH_3COOH/H_2O, 100:11:11:26]. The flavonoid containing fraction (65 mg) was further subjected to MPLC (Li Chroprep RP-18) and eluted with increasing concentrations of MeOH in H_2O (20 to 70%). Repeated MPLC (Li Chroprep RP-18, MeOH/H_2O, 50:50) of selected fractions yielded mixture of *cis*- and *trans*-tiliroside (**1** and **2**, 4.8 mg), rhodiolgin (**3**, 0.8 mg), hyperoside (**4**, 10.8 mg), isoquercetrin (**5**, 2.2 mg), miquelianin (**6**, 4.9 mg), sinocrassoside D_2 (**7**, 1.2 mg) and gossypetin-3-*O*-β-D-galactopyranosyl-7-*O*-α-L-rhamnopyranoside (**8**, 5.9 mg). Known compounds were identified by comparison of their spectral data with those reported in the literature and by comparison with authentic samples. It should be noted that the isolated amounts do not represent the real concentration of the compounds in the plant.

Gossypetin 3-*O*-β-D-galactopyranoside-7-*O*-α-L-rhamnopyranoside (**8**)

Yellowish amorphous powder.
UV λ_{max} nm (MeOH): 260, 275, 303sh, 352; + NaOMe 267, 339sh, 398; + $AlCl_3$ 277, 315sh, 452; + $AlCl_3$ / HCl 249sh, 282, 312sh, 365, 429; + NaOAc 270, 336, 390.
^{1}H NMR (CD_3OD): Table 2.
^{13}C NMR (CD_3OD): Table 2.
Negative HR-ESI-TOF-MS: *m/z* 625.14035 (calcd. for $C_{27}H_{29}O_7$ 625.14102) $[M-H]^+$

Acid hydrolysis: Flavonoid **8** (2 mg) was dissolved in MeOH (5 mL) and refluxed with 8% HCl (2 mL) for 2 h. The reaction mixture was evaporated to dryness, dissolved in H_2O (2 mL) and neutralized with NaOH. The neutralized product was analyzed by TLC (silica gel, *n*-PrOH-EtOAc-H_2O, 7:2:1) in the presence of authentic samples.

Free radical scavenging activity of the DPPH radical: The DPPH radical scavenging method was used for determination of the antioxidant capacity of the extracts and individual compounds [9,10]. Different concentrations of the extracts (10, 20, 50, 100 and 200 μg/mL in MeOH) and individual compounds (5, 10, 20 and 50 μg/mL in MeOH) were added in equal volume (2.5 mL) to the MeOH solution of DPPH$^\bullet$ (0.3 mM, 1 mL). After 30 min at room temperature, the absorption values were measured at 517 nm on a spectrophotometer (Jenway 6320D) and converted into the percentage antioxidant activity using the following equation: DPPH$^\bullet$ anti-radical scavenging capacity (%) = $[1-(A_{sample} - A_{blank})/A_{control}]x100$. MeOH (1.0 mL). The solution plus plant extract solution (2.5 mL) was used as a blank, while DPPH$^\bullet$ solution plus MeOH was used as a control. The extracts were measured in triplicate on 2 different days. The results are presented as a mean ± SD. The IC_{50} values were calculated by Software Prizm 3.00.

Acknowledgements - The authors are thankful to Dr Milka Jadranin (University of Belgrade, Faculty of Chemistry, Belgrade) for preparing the HRMS. This work was supported by the National Science Fund, Ministry of Education, Youth and Science, Bulgaria, project DTK 02/38 (2009).

References

[1] Kurtto A, Fröhner SE, Lampinen R. (Eds) (**2007**) *Atlas Florae Europaeae. Distribution of Vascular Plants in Europe*. Vol. ***14***. *Rosaceae* (*Alchemilla* and *Aphanes*). The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo, Helsinki. pp.200.

[2] (a) Biological Diversity Act. State Gazette, №77/02.08. 2002. (in Bulgarian); (b) Vitkova A. (**2009**) *Alchemilla mollis* (Buser) Rothm. In *Red list of Bulgarian vascular plants*. Petrova A, Vladimirov V. (Eds). *Phytologia Balcanica, **15***, 63-94; (c) Vitkova A. (**2011**) *Alchemilla mollis* (Buser) Rothm. In *Red Book of R Bulgaria*. Vol. 1. Plants, Peev D. (Ed.) In press.

[3] Vitkova A. (**1997**) Contribution to biological investigations of *Alchemilla mollis* (Buser) Rothm. *Phytologia Balcanica*, ***3***, 57-61.

[4] Vitkova A, Gavrilova A, Tashev A. (**2011**). *Alchemilla mollis* (Rosaceae) – critically endangered species in Bulgaria. *Phytologia Balcanica*, in press.

[5] Ivanov I, Landgev I, Neshev G. (**1977**) *Bilkite v Bulgaria i izpolzvaneto im* (Medicinal plants in Bulgaria and their applications), Sofia. 256-257.

[6] Bisset NG. (**1994**) Lady's Mantle In *Herbal Drugs and Phytopharmaceuticals*. Wichtl M and NG Bisset (Eds), Medpharm Scientific Publishers, Stuttgart. 52-54.

[7] (a) Lamaison JL, Carnat A, Petitjean-Freytet C. (**1990**) Tannin content and inhibiting activity of elastase in Rosaceae. *Annales Pharmaceutiques Francaises*, ***48***, 335-340; (b) Filipek J. (**1992**) Effect of *Alchemilla xanthochlora* water extracts on lipid peroxidation and superoxide anion scavenging activity. *Pharmazie*, ***47***, 717-718; (c) Jonadet M, Meunier MT, Villie F, Bastide JP, Lamaison JL. (**1986**) Flavonoids extracted from *Ribes nigrum* L. and *Alchemilla vulgaris* L.: 1. *In vitro* inhibitory activities on elastase, trypsin and chymotrypsin. 2. Angioprotective activities compared *in vivo*. *Journal of Pharmacology*, ***17***, 21-27; (d) Schimmer O, Lindenbaum M. (**1995**) Tannins with antimutagenic properties in the herb of *Alchemilla* species and *Potentilla anserine. Planta Medica*, ***61***, 141-145.

[8] (a) Tuka L, Tamas M. (**1977**) Determination of flavonoids in *Alchemilla mollis* (Buser) Rothm and *Alchemilla* L. (Lady's mantle), *Farmacia*, ***25***, 247-252; (b) Vitkova A. (**1996**) A comparative assessment and dynamics of accumulation of flavonoids and tannins in species of *Alchemilla* L. (Rosaceae). *Fitology*, ***48***, 11-18; (c) Ayaz F A, Hayirlioglu-Ayaz S. (**2001**) Total phenols and condensed tannins in the leaves of some *Alchemilla* species. *Biologia*, ***6***, 449-453.

[9] Cimpoiu C. (**2006**) Analysis of some natural antioxidants by thin–layer chromatography and high performance thin-layer chromatography. *Journal of Liquid Chromatography & Related Technologies*, ***29***, 1125-1142.

[10] Matkowski A, Piotrowska M. (**2006**) Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. *Fitoterapia*, ***77***, 346-353.

[11] Matlawska I, Sikorska M, Bylka W. (**1999**) Flavonoid compounds in *Lavatera thuringiaca* L. (Malvaceae) flowers. *Acta Poloniae Pharmaceutica*, ***56***, 453-445.

[12] Zapesochnaya GG, Kurkin VA, Shchavlinskii AN. (**1985**) Flavonoids of the epigeal part of *Rhodiola rosea*. II. Structures of new glycosides of herbacetin and of gossypetin. *Chemistry of Natural Compounds*, ***21***, 464-473**.**

[13] Tatsis EC, Boeren S, Exarchou V, Troganis AN, Vervoort J, Gerothanassis IP. (**2007**) Identification of the major constituents of *Hypericum perforatum* by LC/SPE/NMR and LC/MS. *Phytochemistry*, ***68***, 383-393.

[14] Morikawa T, Xie H, Wang T, Matsuda H, Yoshikawa M. (**2008**) Bioactive constituents from Chinese natural medicines. XXXII.1) Aminopeptidase N and aldose reductase inhibitors from *Sinocrassula indica*: Structures of sinocrassosides B_4, B_5, C_1, and D_1—D_3. *Chemical & Pharmaceutical Bulletin*, ***56***, 1438-1444.

[15] Zheng CD, Li G, Li HQ, Xu XJ, Gao JM, Zhang AL. (**2010**) DPPH-scavenging activities and structure-activity relationships of phenolic compounds. *Natural Product Communications*, ***5***, 1759-1765**.**

[16] Felser C, Schimmer O. (**1999**) Flavonoid glycosides from *Alchemilla speciosa*, *Planta Medica*, ***65***, 668-670.

[17] Lamaison JL, Carnat A, Petitjean-Freytet C, Carnat AP. (**1991**) Quercetin-3-glucuronide, the main flavonoid of Lady's Mantle, *Alchemilla xanthochlora* Rothm. (Rosaceae). *Annales Pharmaceutiques Francaises*, ***49***, 186-189.

[18] Fraisse D, Heitz A, Carnat A, Carnat A-P, Lamaison J-L. (**2000**) Quercetin 3-arabinopyranoside, a major flavonoid compound from *Alchemilla xanthochlora*. *Fitoterapia*, ***71***, 463-464.

[19] D'Agostino M, Dini I, Ramundo E, Senatore F. (**1998**) Flavonoid glycosides of *Alchemilla vulgaris* L. *Phytotherapy Research*, ***12***, (Suppl. 1), S162–S163.

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 *Alpinia officinarum* against Respiratory Syncytial Virus, Poliovirus, Measles Virus, and Herpes Simplex Virus Type 1 *in vitro*
Katsuhiko Konno, Rie Sawamura, Yi Sun, Ken Yasukawa, Tomomi Shimizu, Wataru Watanabe, Masahiko Kato, Ryuichi Yamamoto and Masahiko Kurokawa **1881**

Isolation of *C*-glycosyl Xanthones from *Coffea pseudozanguebariae* and Their Location
Pascale Talamond, Geneviève Conejero, Jean-Luc Verdeil and Jean-Luc Poëssel **1885**

Antifungal Activity and Isomerization of Octadecyl *p*-coumarates from *Ipomoea carnea* subsp. *fistulosa*
Eugene Sebastian J. Nidiry, Girija Ganeshan and Ankanahalli N. Lokesha **1889**

New Glucose Esters from the Fresh Leaves of *Jacaranda mimosaefolia*
Christianah A. Elusiyan and Tiwalade A. Olugbade **1893**

Shamiminol: A New Aromatic Glycoside from the Stem Bark of *Bombax ceiba*
Shaheen Faizi, Sadia Zikr-Ur-Rehman and Muhammad Ali Versiani **1897**

Two New Phenolic Glycosides from *Viburnum plicatum* var. *plicatum* f. *plicatum*
Saki Katagiri, Yoshiki Watanabe, Yasunori Yaoita, Masao Kikuchi and Koichi Machida **1901**

Antimicrobial Chemical Constituents from the Endophytic Fungus *Phomopsis* sp. from *Notobasis syriaca*
Hidayat Hussain, Michel Kenne Tchimene, 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 *Phomopsis* sp. from *Ligustrum vulgare*
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 *Alternaria* 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 *Myrothecium verrucaria* 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 Medellin-Milán and Alejandro García-Carrancá **1925**

Chemometrics Evaluation of the Herbal Drug *Andrographis paniculata*
Shiv Narayan Sharma, Zenu Jha and D. K. Sharma **1929**

***Garcina cambogia* Leaf and Seawater for Tannase Production by Marine *Aspergillus awamori* 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 *Thelechitonia trilobata* Growing in South Africa
Jamie Peebles, Ephraim Gwebu, Opeoluwa Oyedeji, Sarah Nanyonga, Nokuthula Kunene, David Jackson, William Setzer and Adebola Oyedeji **1945**

Chemical Composition and Antibacterial Activity of Essential oil from *Salvia mukerjeei*
Lalit Mohan, Anuradha Negi, Anand B. Melkani and Vasu Dev **1949**

Review/Account

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

Original Paper ***Page***

Sibiralactone: A New Monoterpene from *Sibiraea angustata*
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 *S*-(-)-Perillyl Alcohol by *Fusarium heterosporium*
Ismail Kiran **1805**

A Phytochemical Investigation of *Zanthoxylum setulosum*
Tameka M. Walker, Bernhard Vogler, Debra M. Moriarity, William A. Haber and William N. Setzer **1807**

Cytotoxic Cembranoids from the Red Sea Soft Coral *Sarcophyton glaucum*
Mohamed-Elamir F. Hegazy, Ahmed A. El-Beih, Alaa Y. Moustafa, Abdelhamed A. Hamdy, Montaser A. Alhammady, Rehab M. Selim, Mohamed Abdel-Rehim and Paul W. Paré **1809**

C-Lactam Derivatives of Oleanolic 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 C_{30} Columns
Daniele Giuffrida, Paola Dugo, Giacomo Dugo, Germana Torre and Luigi Mondello **1817**

New Antifungal Cholestane and Aldehyde Derivatives from the Red Alga *Laurencia papillosa*
Walied M. Alarif, Sultan S. Al-Lihaibi, Ahmed Abdel-Lateff and Seif-Eldin N. Ayyad **1821**

Steroidal Saponins from the Fruits of *Cestrum ruizteranianum*
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 *Nerine bowdenii*
Lucie Cahlíková, Stanislav Zavadil, Kateřina Macáková, Irena Valterová, Andrea Kulhánková, Anna Hošťálková, Jiří Kuneš and Lubomír Opletal **1827**

HPLC Determination of Majdine in *Vinca herbacea*
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 *Derris indica* (*Millettia pinnata*) and *Caesalpinia crista*
Yuling Yin, Hamako Sasamoto and Hiroshi Ashihara **1835**

Anti-adipogenic Activity of *Cordyceps militaris* 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 *Xylaria* sp. 101
Yao-Yao Li, Zhi-Yu Hu, and Yue-Mao Shen **1843**

A Novel Flavonoid and Furoquinoline Alkaloids from *Vepris glomerata* and their Antioxidant Activity
Joyce J. Kiplimo, Md. Shahidul Islam and Neil A. Koorbanally **1847**

Flavonoid Constituents and Free Radical Scavenging Activity of *Alchemilla mollis*
Antoaneta Trendafilova, Milka Todorova, Milena Nikolova, Anna Gavrilova and Antonina Vitkova **1851**

Ultrasound-assisted Extraction of Total Phenols and Flavonoids from Dry Tobacco (*Nicotiana tabacum*) Leaves
Ivana T. Karabegović, Vlada B. Veljković and Miodrag L. Lazić **1855**

Characterization of Polyphenolic Compounds in Unripe Chinotto (*Citrus myrtifolia*) 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
Ângelo Luís, Fernanda Domingues and Ana Paula Duarte **1863**

HPLC/PDA/ESI-MS Evaluation of Saffron (*Crocus sativus L.*) Adulteration
Leonardo Sabatino, Monica Scordino, Maria Gargano, Adalgisa Belligno, Pasqualino Traulo and Giacomo Gagliano **1873**

Continued inside backcover