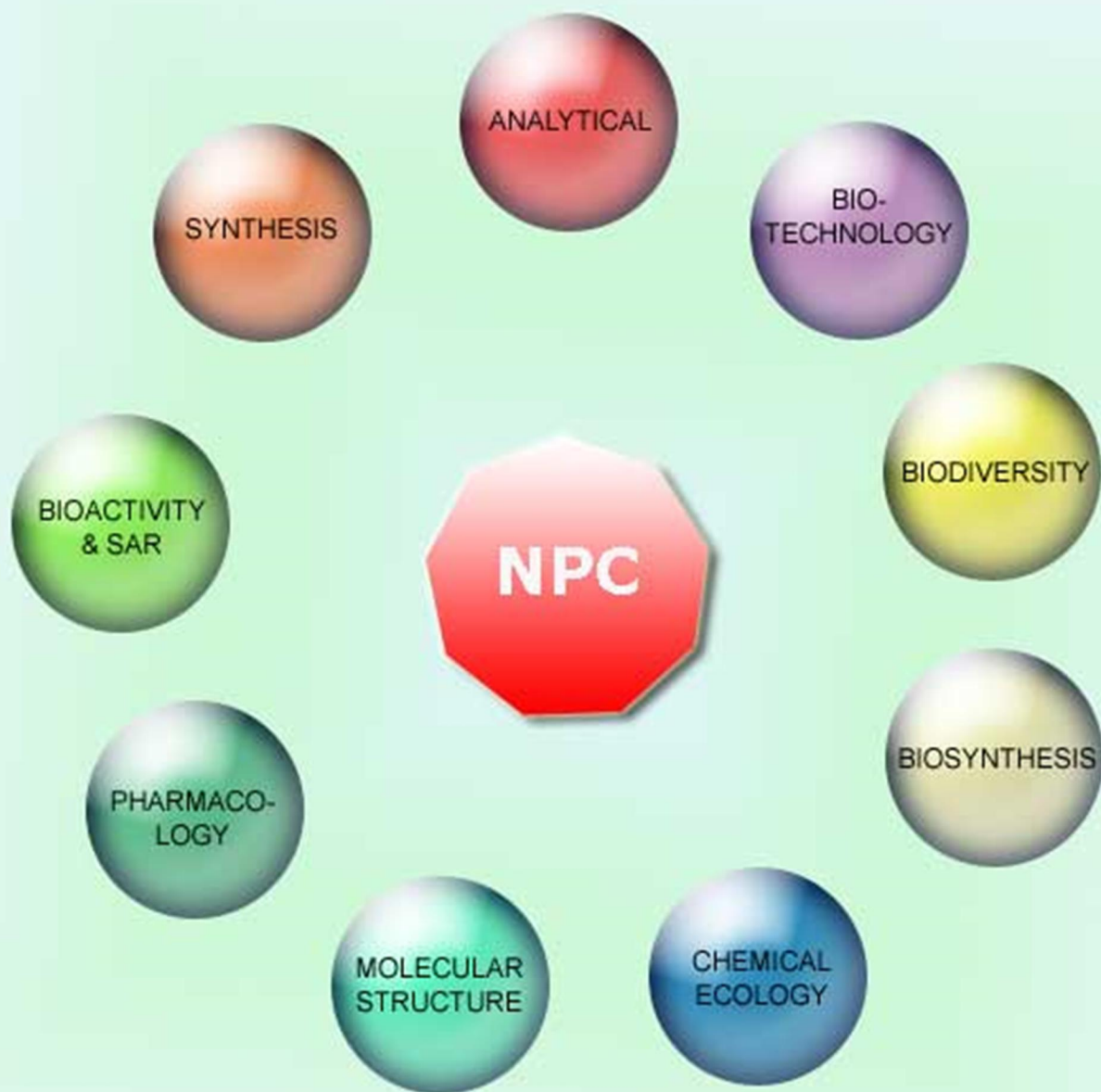


NATURAL PRODUCT COMMUNICATIONS

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



Volume 9. Issue 4. Pages 445-596. 2014
ISSN 1934-578X (printed); ISSN 1555-9475 (online)
www.naturalproduct.us

EDITOR-IN-CHIEF**DR. PAWAN K AGRAWAL**

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

EDITORS**PROFESSOR ALEJANDRO F. BARRERO**

Department of Organic Chemistry,
University of Granada,
Campus de Fuente Nueva, s/n, 18071, Granada, Spain
afbarre@ugr.es

PROFESSOR ALESSANDRA BRACA

Dipartimento di Chimica Bioorganica e Biofarmacia,
Università 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

Faculty of Pharmaceutical Sciences
Hokuriku University
Ho-3 Kanagawa-machi, Kanazawa 920-1181, Japan
y-tezuka@hokuriku-u.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

HONORARY EDITOR**PROFESSOR GERALD BLUNDEN**

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

ADVISORY BOARD

Prof. Viqar Uddin Ahmad
Karachi, Pakistan

Prof. Giovanni Appendino
Novara, Italy

Prof. Yoshinori Asakawa
Tokushima, Japan

Prof. Roberto G. S. Berlinck
São Carlos, Brazil

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. Fatih Demirci
Eskişehir, Turkey

Prof. Dominique Guillaume
Reims, France

Prof. Ana Cristina Figueiredo
Lisbon, Portugal

Prof. Cristina Gracia-Viguera
Murcia, Spain

Prof. Duvvuru Gunasekar
Tirupati, India

Prof. Hisahiro Hagiwara
Niigata, Japan

Prof. Kurt Hostettmann
Lausanne, Switzerland

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

Prof. Leopold Jirovetz
Vienna, Austria

Prof. Vladimir I Kalinin
Vladivostok, Russia

Prof. Niel A. Koorbanally
Durban, South Africa

Prof. Chiaki Kuroda
Tokyo, Japan

Prof. Hartmut Laatsch
Göttingen, Germany

Prof. Marie Laccaille-Dubois
Dijon, France

Prof. Shoen-Sheng Lee
Taipei, Taiwan

Prof. Imre Mathe
Szeged, Hungary

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. Luca Rastrelli
Fisciano, Italy

Prof. Stefano Serra
Milano, Italy

Prof. Monique Simmonds
Richmond, UK

Dr. Bikram Singh
Palampur, India

Prof. John L. Sorensen
Manitoba, Canada

Prof. Johannes van Staden
Scottsville, South Africa

Prof. Valentin Stonik
Vladivostok, Russia

Prof. Winston F. Tinto
Barbados, West Indies

Prof. Sylvia Urban
Melbourne, Australia

Prof. Karen Valant-Vetschera
Vienna, Austria

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. 2013 subscription price: US\$2,395 (Print, ISSN# 1934-578X); US\$2,395 (Web edition, ISSN# 1555-9475); US\$2,795 (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.

Cytotoxicity and Antimicrobial Activity of the Essential Oil from *Satureja montana* subsp. *pisidica* (Lamiaceae)

Tatjana Kundaković^{a*}, Tatjana Stanojković^c, Branka Kolundžija^c, Stevan Marković^e, Branka Šukilović^e, Marina Milenković^b and Branislava Lakušić^d

^aDepartment of Pharmacognosy, University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia

^bDepartment of Immunology and Microbiology, University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia

^cInstitute for Oncology and Radiology of Serbia, 11000 Belgrade, Serbia

^dDepartment of Botany, University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia

^eUniversity of Belgrade, Faculty of Pharmacy, Belgrade, Serbia

ktatjana@pharmacy.bg.ac.rs

Received: March 21st, 2013; Accepted: January 13th, 2014

The antimicrobial and cytotoxic activities of the essential oil of *Satureja montana* ssp. *pisidica* from two localities (mountains Korab and Galičica) were studied. Forty-nine components were identified in the each sample. Oxygenated monoterpene hydrocarbons were the major compounds: carvacrol, thymol, carvacrol methyl ether and β -linalool. Both tested essential oils showed very high and similar antimicrobial activity. Minimal inhibitory concentrations ranged from 12.5 μ g/mL against *S. epidermidis* to 50 μ g/mL against *P. aeruginosa* and *C. albicans*. The cytotoxic effect of the essential oils was tested against MDA-MB-361, MDA-MB-453, HeLa, LS174 and MRC5 cells. The essential oil from Korab demonstrated significantly better results than the oil from Galičica, particularly against HeLa and MDA-MB-453 cell lines, with IC₅₀ values of 63.5 and 72.3 μ g/mL, while the oil from Galičica was the most active on the human epithelial cervical cancer HeLa cells (IC₅₀ 99.7 μ g/mL).

Keywords: *Satureja montana* ssp. *pisidica*, Essential oil, Cytotoxicity, Antimicrobial activity, Carvacrol.

The genus *Satureja* L. includes over 30 species of herbs and shrubs, often aromatic, with a centre of distribution in the Mediterranean Basin. In the area of the Balkan Peninsula nine species of this genus have been registered. *S. montana* contains three subspecies: ssp. *montana*, ssp. *variegata* (Host) P.W.Ball, and ssp. *pisidica* (Wettst.) Šilić [1].

S. montana ssp. *pisidica* (syn. *S. macedonica* Formanek, *S. montana* L. var. *pisidica* (Wettst.) Hal., *S. olympica* Hal.) is widespread in Macedonia (FYRM), but sporadically in Serbia and Montenegro. The chemistry of *S. montana* ssp. *montana* essential oil has been well-studied [2,3] and several biological activities, like antimicrobial, antiviral, antiparasitic, immunostimulative and antioxidative have been shown [3-7]. Research data for the essential oil composition of *S. montana* ssp. *pisidica* are scarce, while the biological significance of its essential oil has not yet been investigated [2,7]. This paper aims to describe the composition of the essential oils of *S. montana* ssp. *pisidica* from two localities, as well their antimicrobial properties and cytotoxicity against five cancer cell lines.

The aerial parts, before the flowering period, of *S. montana* ssp. *pisidica* yielded a moderate content of essential oil (0.9%, v/w; Korab; 1.1%, v/w; Galičica; light yellow calculated on dry weight basis). The chemical composition of the oils from the two localities is summarized in Table 1. Forty-eight components were identified in the samples of the essential oils, representing almost 100% of the oil (Korab: 94.1%; Galičica: 98.7% respectively). The composition of the oils obtained from the different localities was qualitatively the same but some quantitative differences could be seen.

Oxygenated monoterpene hydrocarbons were the major compounds (Korab: 58.4%; Galičica: 80.3% respectively). Carvacrol was the most dominant compound in both oils (Korab: 20.9%; Galičica:

37.6%, respectively). Also, a high content of thymol was determined in the essential oil from Galičica. Carvacrol methyl ether was detected in very high content in the sample from Korab (11.8%), as well as *p*-cymene (17.1%), but in very low content in the essential oil from the plant from Galičica (0.5% and 6.8%, respectively). γ -Terpinene was identified in both samples (5-8%). Sesquiterpene hydrocarbons (Korab: 8.8%; Galičica: 5.7%) and oxygenated sesquiterpenes (Korab: 7.0%; Galičica: 1.6%) was low in both samples. Spathulenol and β -caryophyllene were the most abundant sesquiterpenes. The major difference in composition between the two oils was in the content of thymol, carvacrol methyl ether and β -linalool.

The only paper dealing with the composition of the essential oil of *S. montana* ssp. *pisidica* was that of Slavkowska *et al.* from the locality of Galičica, but with *p*-cymene as a major compound (29.3%), followed by a high content of linalool (24%) and carvacrol (18.3%) [2]. This could be explained by the fact that the plant was collected in the flowering period, while our samples were collected before flowering. Such differences in the amounts of the main components in the essential oil, depending on the stage of plant development, were found for *S. cuneifolia* Ten. [8], *S. montana* L., *S. subspicata* Bartl. ex Vis. [9] and *S. horvatii* Šilić [10].

The antimicrobial activity results of *S. montana* ssp. *pisidica* essential oils, presented in Table 2, are expressed as minimal inhibitory concentrations (MICs). These are the first results of antimicrobial activity of essential oil of *S. montana* ssp. *pisidica*. Both tested essential oils showed very high and similar antimicrobial activity, no matter the difference in carvacrol and thymol contents. The MIC ranged from 12.5 μ g/mL against *S. epidermidis* (Galičica) to 50 μ g/mL against *P. aeruginosa* and *C. albicans* (Korab). The essential oil from Galičica showed slightly better activity, especially against *S. epidermidis*, which could be

Table 1: Composition of essential oils of *Satureja montana* ssp. *pididica*.

| Components | Area (%) | | RI exp ^a |
|--------------------------------|----------|----------|---------------------|
| | Korab | Galičica | |
| α -Thujene | 0.6 | 0.4 | 930 |
| α -Pinene | 0.4 | 0.3 | 937 |
| Camphene | 0.2 | 0.2 | 952 |
| 1-octen-3-ol | - | 0.8 | 975 |
| Sabinene | 0.1 | - | 976 |
| β -Pinene | 0.2 | - | 980 |
| β -Myrcene | 0.6 | 0.8 | 992 |
| α -Phellandrene | 0.1 | 0.1 | 1006 |
| α -Terpinene | 0.6 | 1.4 | 1018 |
| <i>p</i> -Cymene | 17.1 | 6.8 | 1032 |
| (-)-limonene | - | 0.3 | 1033 |
| 1,8-Cineol | 0.3 | 0.1 | 1035 |
| (<i>Z</i>)- β -Ocimene | 1.6 | 0.9 | 1037 |
| (<i>E</i>)- β -Ocimene | 0.3 | 0.5 | 1047 |
| γ -Terpinene | 5.0 | 8.2 | 1063 |
| <i>cis</i> -Sabinene hydrate | 0.7 | 1.4 | 1070 |
| <i>cis</i> -Linalool oxide | 0.1 | 0.3 | 1074 |
| α -Terpinolene | 0.2 | 0.2 | 1090 |
| β -Linalool | 15.2 | 0.6 | 1112 |
| <i>endo</i> -Borneol | 1.3 | 1.3 | 1170 |
| Terpinene-4-ol | 0.5 | 1.5 | 1181 |
| α -Terpineol | - | 0.3 | 1189 |
| Thymol methyl ether | - | 1.9 | 1236 |
| <i>p</i> -Cymen-8-ol | 0.1 | - | 1194 |
| Carvacrol methyl ether | 11.8 | 0.5 | 1251 |
| (+)-Carvone | 0.1 | - | 1259 |
| Thymol | 0.2 | 24.5 | 1290 |
| Carvacrol | 20.9 | 37.6 | 1295 |
| Thymol acetate | - | 0.2 | 1359 |
| Carvacrol acetate | 0.1 | 0.3 | 1379 |
| α -Copaene | 0.1 | - | 1384 |
| β -Caryophyllene | 3.3 | 3.5 | 1428 |
| β -Copaene | 0.1 | - | 1436 |
| Aromadendrene | 0.1 | 0.3 | 1446 |
| α -Humulene | 0.1 | 0.1 | 1462 |
| Aloaromadendren | 0.2 | - | 1470 |
| γ -Muurolene | 0.1 | - | 1487 |
| Germacrene D | 1.2 | - | 1493 |
| α -Amorphene | - | 0.1 | 1490 |
| Viridiphlorene | - | 0.4 | 1501 |
| Bicyclogermacrene | 1.7 | - | 1509 |
| β -Bisabolene | 1.5 | 0.9 | 1520 |
| γ -Cadinene | 0.1 | 0.1 | 1521 |
| δ -Cadinene | 0.3 | 0.3 | 1532 |
| Spathulenol | 4.0 | 0.5 | 1589 |
| Caryophyllene oxide | 2.3 | 1.1 | 1592 |
| <i>iso</i> -Spathulenol | 0.5 | - | 1621 |
| α -Cadinol | 0.2 | - | 1669 |
| Grouped components | | | - |
| Monoterpene hydrocarbons | 19.9 | 11.2 | |
| Oxygenated monoterpenes | 58.4 | 80.2 | |
| Sesquiterpene hydrocarbons | 8.8 | 5.7 | |
| Oxygenated sesquiterpenes | 7.0 | 1.6 | |
| Other compounds | - | - | |
| Total (%) | 94.1 | 98.8 | |

^aRI_{exp}-Retention indices relative to C₉-C₂₃ *n*-alkanes on HP 5MS.

attributed to a high content of phenolic monoterpenes. As the essential oils from *Satureja* species are known as antimicrobial agents, our results were in accordance with the most recent paper of Marin *et al.* [3], as well as with others [9,11].

Carvacrol, a monoterpene phenol present in very high content in the tested essential oils, possesses a wide spectrum of antimicrobial activity, extended to food born pathogenic fungi, yeasts and bacteria. The mechanism of antimicrobial activity could be connected with the lipophilicity of carvacrol and its effects on the structural and functional properties of the cytoplasmatic membrane [12].

To determine the cytotoxic effect of the essential oils, MDA-MB-361 (estrogen-dependant) and MDA-MB-453 (estrogen-nondependant) breast cancer cell lines, a human epithelial cervical cancer cell HeLa, a human colon cancer cell line LS174, as well as healthy MRC-5 human embryonic lung fibroblast cell lines were treated with compounds, and cell survival was determined using the

Table 2: Antimicrobial activity of *Satureja montana* ssp. *pididica* essential oil.

| Microorganisms | MIC (μ g/mL) | | | | |
|----------------------------------------------|----------------------------------------|----------|------------|----------|----------|
| | <i>S. montana</i> ssp. <i>pididica</i> | | Ampicillin | Amikacin | Nystatin |
| | Korab | Galičica | | | |
| <i>Staphylococcus aureus</i> ATCC 25923 | 25.0 | 25.0 | 0.5 | n.t.* | n.t. |
| <i>Staphylococcus epidermidis</i> ATCC 12228 | 25.0 | 12.5 | 1.5 | n.t. | n.t. |
| <i>Micrococcus luteus</i> ATCC 3341 | 25.0 | 25.0 | 2.0 | n.t. | n.t. |
| <i>Bacillus subtilis</i> ATCC 6633 | 25.0 | 25.0 | 1.8 | n.t. | n.t. |
| <i>Escherichia coli</i> ATCC 25922 | 25.0 | 25.0 | 2.0 | 1.5 | n.t. |
| <i>Klebsiella pneumoniae</i> ATCC 13883 | 25.0 | 25.0 | 2.8 | 2.0 | n.t. |
| <i>Pseudomonas aeruginosa</i> ATCC 27853 | 50.0 | 25.0 | n.t. | 2.5 | n.t. |
| <i>Candida albicans</i> ATCC 10231 | 50.0 | 25.0 | n.t. | n.t. | 3.8 |
| <i>Candida albicans</i> ATCC 10259 | 50.0 | 25.0 | n.t. | n.t. | 4.2 |

*n.t.- not tested

MTT assay. The cytotoxicity of the oils on human cancer cell lines is shown in Figure 1 and the IC₅₀ values are given in Table 3.

Essential oil from Galičica was the most active on HeLa cancer cells (IC₅₀ 99.7 μ g/mL), with lower activity against the other cell cultures. The essential oil from Korab demonstrated significantly better results, particularly for HeLa and MDA-MB-453 cell lines (IC₅₀ 63.5 and 72.3 μ g/mL). Also, it was observed that at concentrations of 100 μ g/mL and higher (Korab), or at 200 μ g/mL (Galičica), the essential oils induced a dramatic drop in the survival of malignant cells (Figure 1.).

The *S. montana* ssp. *pididica* essential oil from Galičica showed no adverse effect on the MRC-5 cell line (IC₅₀ 297.4 μ g/mL). In contrast, the sample from Korab displayed a comparable IC₅₀ value on malignant and MRC 5 cells (Table 3). The small differences between IC₅₀ values led to our conclusion that the sample from Korab, due to a cytotoxic effect on healthy MRC-5 cells, needs further consideration for its toxicity. The cytotoxicity of *S. montana* essential oil has not been studied before, but the cytotoxicity of essential oils rich in carvacrol, as well as carvacrol itself has been studied in detail. Četojević-Simin *et al.* showed that the methanol extract of *S. montana* stimulated proliferation of HT-29 cells, and inhibited proliferation of HeLa cells with no activity against MCF-7 cells [13].

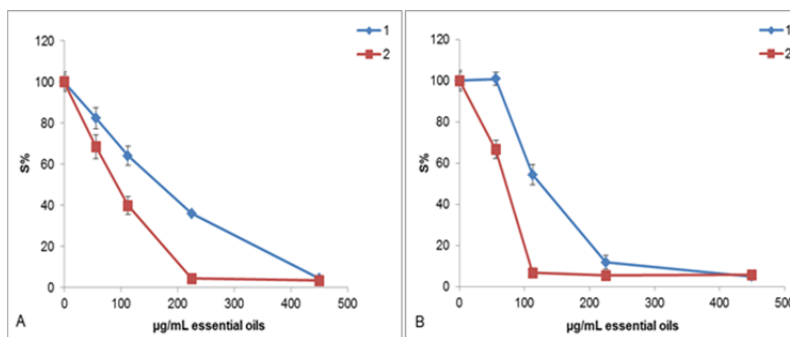
Interestingly, as carvacrol was recognized as a major and lipophilic compound, the essential oil with less carvacrol (Korab) possessed higher cytotoxic activity against the tested cell lines, which could be attributed to other important monoterpenes like *p*-cymene, γ -terpinene and β -linalool. A recent paper by Yousefzadi *et al.* has shown the significant antiproliferative effects of *S. sahendica* essential oil rich in thymol, γ -terpinene and *p*-cymene against MCF7, Vero, SW480 and JET 3 cell lines, in a dose-dependent manner [14].

Several studies have shown significant cytotoxic activity of carvacrol against A549 cell line [15], and myoblast cells, even after activation of mutated N-ras oncogene [16], human metastatic breast cancer cells MDA-MB 231 [17], as well anti-proliferative and anti-carcinogenic activity *in vivo* [18]. A recent study by Liang and Lu

Table 3: Cytotoxicity of the essential oils of *Satureja montana* ssp. *pisidica* from Korab and Galičica against MDA-MB 361, MDA-MB-453, HeLa, LS174 and MRC5 cell lines (expressed as IC₅₀).

| Essential oil | IC ₅₀ (µg/mL)* | | | | |
|---------------------------------------------------|---------------------------|------------|-----------|------------|------------|
| | MDA-MB-361 | MDA-MB-453 | HeLa | LS174 | MRC5 |
| <i>S. montana</i> ssp. <i>pisidica</i> (Galičica) | 234.6±0.11 | 240.3±0.31 | 99.7±0.11 | 189.8±0.31 | 297.4±0.11 |
| <i>S. montana</i> ssp. <i>pisidica</i> (Korab) | 109.0±0.21 | 72.3±0.11 | 63.5±0.31 | 99.4±0.22 | 102.8±0.11 |

*IC₅₀ values were expressed as the mean±SD determined from the results of MTT assay in three independent experiments.

**Figure 1:** Representative graphs: the dose-dependent cytotoxic effect on (A) MDA-MB-361 cell line and (B) MDA-MB-453 cell line of the essential oils of *S. montana* ssp. *pisidica* from Galičica (1) and Korab (2).

has shown that carvacrol is cytotoxic to human glioblastoma cells in a concentration-dependent manner, influences Ca²⁺ rise, as well as the production of reactive oxygen species (ROS) in human glioblastoma cells [19]. The authors concluded that carvacrol induced cell death through apoptosis mediated by ROS. Similar results were obtained by Hsu *et al.* for thymol, the second major compound in the essential oil of *S. montana* from Galičica [20]. Huang *et al.* tested the anti-proliferative effects of carvacrol on MDA-MB 231 cells, and showed induction of apoptosis in MDA-MB 231 cells with an IC₅₀ of 100 µM [21].

In conclusion, our results have shown strong antimicrobial and cytotoxic activities, which could be attributed to the major phenolic monoterpene, carvacrol. Concerning the difference in cytotoxicity of the two tested oils against healthy human fibroblast cell line MRC-5 with IC₅₀ values of 297.4 µg/mL (Galičica) and 102.8±0.11 (Korab), as well as recent findings of apoptotic effects of thymol and carvacrol, the cytotoxicity to MRC-5 cells needs further research on the underlying molecular mechanisms of action.

Experimental

Plant material: The aerial parts, before the flowering period, of *S. montana* ssp. *pisidica* were collected from mountains Korab (1370 m a. s. l.), and Galičica (1596 m a. s. l.) (FYRM) in July 2011. A voucher specimen was deposited at the Department of Botany, University of Belgrade, Faculty of Pharmacy, Belgrade, Serbia.

Isolation of the essential oil: The plant material was air-dried at room temperature for 3 days and the oil isolated (Korab; 50 g; Galičica: 58 g) by hydrodistillation for 2 h using a Clevenger-type apparatus, according to the Ph. Eur. 6.0 [22].

Essential oil analysis: Volatile constituents were determined by GC and GC-MS. GC analysis was performed on an Agilent 6890N GC system equipped with 5975 MSD and FID, using a HP-5 MS column (30 m x 0.25 mm x 0.25 µm). Injection volume was 2 µL and injector temperature was 200°C with a 10:1 split ratio. Helium was the carrier gas at a flow rate of 1.0 mL/min (constant flow mode). Column temperature was linearly programmed in the range 60-280°C at a rate of 3°C/min and held at 280°C for 5 min. The transfer line was heated at 250°C. The FID detector temperature was 300°C. EI mass spectra (70 eV) were acquired in the m/z range

35-550. Identification of the compounds was based on comparison of their retention indices (RI), their retention times (t_R) and mass spectra with those obtained from authentic samples and/or data bases and literature [23]. Relative percentages of the identified compounds were computed from the GC-FID peak area.

Antimicrobial activity: The antimicrobial activity was evaluated using 7 different laboratory control strains of bacteria: *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *Micrococcus luteus* (ATCC 9341), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), and two strains of yeast *Candida albicans* (ATCC 10231 and ATCC 10259). The broth microdilution method was used to determine minimal inhibitory concentrations (MIC_s) of essential oils according to Clinical and Laboratory Standards Institute and procedure given by Kundaković *et al.* [24,25]. Samples of essential oils were dissolved in dimethyl sulfoxide (DMSO) in concentrations of 1.0 mg/mL. All microbial tests were performed in duplicate and 2 positive growth controls were included. Ampicillin, Amikacin and Nystatin were used as standard substances.

Cytotoxicity assay

Cell lines: MDA-MB-361 (estrogen-dependant) and MDA-MB-453 (estrogen-nondependant) breast cancer cell lines, HeLa - human epithelial cervical cancer cells, LS174 - a human colon cancer cell line and MRC-5 human embryonic lung fibroblast cell lines were grown in RPMI-1640 medium (Sigma) at 37°C. Media were supplemented with 10% fetal bovine serum, L-glutamine, and penicillin-streptomycin (Sigma).

Treatment of cell lines: Stock solutions (100 mg/mL) of essential oils, made in DMSO, were dissolved in the corresponding medium to the required working concentrations (400, 200, 100, 50 and 25 µg/mL). The final concentration of DMSO never exceeded 0.5%, which was non-toxic to the cells. Target neoplastic HeLa cells (2000 cells per well), MDA-MB-453 cells (3000 cells per well), MDA-MB-361 (7000 cells per well), and normal human fetal lung fibroblast MRC-5 cells (5000 cells per well) were seeded into 96-well microtiter plates and 24 h later, after cell adherence, 5 different, double diluted, concentrations of investigated compounds, were added to the wells except for the control cells to which a

nutrient medium only was added. The cultures were incubated for 72 h.

Determination of cell survival: The effects of essential oils on cancer cell survival were determined by the MTT test, according to Mosmann [26] with modification by Ohno and Abe [27], 72 h after

addition of the compounds. The detailed procedure was described Stanojković *et al.* [28].

Acknowledgments - The authors are grateful to the Ministry of Science (Grant Nos. 173021 and 175011) for financial support.

References

- [1] Šilić Č. (1979) *Monografija rodova Satureja L., Calamintha Miller, Micromeria Benthams, Acinos Miller i Clinopodium L. u flori Jugoslavije. Zemaljski Muzej BiH, Sarajevo* (in Serbian).
- [2] Slavkowska V, Jancic R, Bojovic S, Milosavljevic S, Djokovic D. (2001) Variability of essential oils of *Satureja montana* L. and *Satureja kitaibelii* Wierzb. ex Heuff. from the central part of the Balkan peninsula. *Phytochemistry*, **57**, 71-76.
- [3] Marin M, Novaković M, Tešević V, Vučković I, Milojević N, Vuković-Gačić B, Marin PD. (2012) Antioxidative, antibacterial and antifungal activity of the essential oil of wild-growing *Satureja montana* L. from Dalmatia, Croatia (Review). *Flavour and Fragrance Journal*, **27**, 216-223.
- [4] Bezić N, Skočibušić M, Dunkić V. (2005) Phytochemical composition and antimicrobial activity of *Satureja montana* L. and *Satureja cuneifolia* Ten. essential oils. *Acta Botanica Croatica*, **64**, 313-322.
- [5] Skočibušić M, Bezić N. (2003) Chemical composition and antiarrhythmic activities of winter savory (*Satureja montana* L.) essential oil. *Pharmaceutical Biology*, **41**, 622-626.
- [6] Ciani M, Menghini L, Mariani F, Pagiotti R, Menghini A, Fatichenti F. (2000) Antimicrobial properties of essential oil of *Satureja montana* L. on pathogenic and spoilage yeasts. *Biotechnology Letters*, **22**, 1007-1010.
- [7] Stoilova I, Bail S, Buchbauer G, Krastanov A, Stoyanova A, Schmidt E, Jirovetz L. (2008) Chemical Composition, Olfactory Evaluation and Antioxidant Effects of the Essential Oil of *Satureja montana* L. *Natural Product Communications*, **3**, 1035-1042.
- [8] Skočibušić M, Bezić N, Dunkić V. (2004) Variability of *Satureja cuneifolia* Ten. essential oils and their antimicrobial activity depending on the stage of development. *European Food Research and Technology*, **218**, 367-371.
- [9] Čavar S, Maksimović M, Šolc ME, Jerković-Mujkić A, Bešta R. (2008) Chemical composition and antioxidant and antimicrobial activity of two *Satureja* essential oils. *Food Chemistry*, **111**, 648-653.
- [10] Lakušić B, Ristić M, Slavkowska V, Milenković M, Lakušić D. (2011) Environmental and Seasonal Impacts on the Chemical Composition of *Satureja horvati* Šilić (Lamiaceae) Essential Oils. *Chemistry and Biodiversity*, **8**, 483-493.
- [11] Nedorostova L, Kloucek P, Kokoska L, Stolcova M, Pulkrabek J. (2009) Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. *Food Control*, **20**, 157-160.
- [12] Nostro A, Papalia T. (2012) Antimicrobial activity of carvacrol: Current progress and future perspectives (Review). *Recent Patents on Anti-Infective Drug Discovery*, **7**, 28-35.
- [13] Četojević-Simin DD, Čanadanović-Brunet JM, Bogdanović GM, Četković GS, Tumbas VT, Djilas SM. (2004) Antioxidative and antiproliferative effects of *Satureja montana* L. extracts. *Journal of B.U.O.N.* **9**, 443-449.
- [14] Yousefzadi M, Riahi-Madvar A, Hadian J, Rezaee F, Rafiee R. (2012) In vitro cytotoxic and antimicrobial activity of essential oil from *Satureja sahendica*. *Toxicological and Environmental Chemistry*, **94**, 1735-1745.
- [15] Koparal AT, Zeytinoglu M. (2003) Effects of carvacrol on a human non-small cell lung cancer (NSCLC) cell line. *A549* (Conference Paper), *Cytotechnology*, **43**, 149-154.
- [16] Zeytinoglu H, Incesu Z, Baser KHC. (2003) Inhibition of DNA synthesis by carvacrol in mouse myoblast cells bearing a human N-ras oncogene. *Phytomedicine*, **10**, 292-299.
- [17] Arunasree KM. (2010) Anti-proliferative effects of carvacrol on a human metastatic breast cancer cell line, MDA-MB 231. *Phytomedicine*, **17**, 581-588.
- [18] Karkabounas S, Kostoula OK, Daskalou T, Veltsistas P, Karamouzis M, Zelovitis I, Metsios A, Lekkas P, Evangelou AM, Kotsis N, Skoufos I. (2006) Anticarcinogenic and antiplatelet effects of carvacrol. *Experimental Oncology*, **28**, 121-125.
- [19] Liang WZ, Lu CH. (2012) Carvacrol-induced [Ca²⁺] rise and apoptosis in human glioblastoma cells. *Life Sciences*, **90**, 703-711.
- [20] Hsu SS, Lin KL, Chou CT, Chiang AJ, Liang WZ, Chang HT, Tsai JY, Liao WC, Huang FD, Huang JK, Chen IS, Liu SI, Kuo CC, Jan CR. (2011) Effect of thymol on Ca²⁺ homeostasis and viability in human glioblastoma cells. *European Journal of Pharmacology*, **670**, 85-91.
- [21] Huang TC, Lin Yting, Chuang KP. (2010) Carvacrol has the priming effects of reactive oxygen species (ROS) production in C6 glioma cells. *Food and Agricultural Immunology*, **21**, 47-55.
- [22] The European Pharmacopeia, 6th Edition, Ph.Eur. 6.0. (2007) Strasbourg: Council of Europe.
- [23] Adams RP (2001) Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy. Allured Publishing: Carol Stream, IL.
- [24] Kundaković T, Milenković M, Zlatković S, Kovačević N, Nikolić G. (2011) Composition of *Satureja kitaibelii* Essential Oil and its Antimicrobial Activity, *Natural Product Communications*, **6**, 1353-1356.
- [25] Clinical and Laboratory Standards Institute (CLSI). (2005) Performance standards for antimicrobial susceptibility testing: 15th informational supplement. *CLSI document M100-S15*. Wayne, PA, USA.
- [26] Mosmann T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, **65**, 55 - 63.
- [27] Ohno M, Abe T. (1991) Rapid colorimetric assays for the quantification of leukemia inhibitory factor (LIF) and interleukin-6 (IL-6). *Journal of Immunological Methods*, **145**, 199-203.
- [28] Stanojković T, Kolundžija B, Ćirić A, Soković M, Nikolić D, Kundaković T. (2013) Cytotoxicity and antimicrobial activity of *Satureja kitaibelii* Wierzb. Ex Heuff (Lamiaceae), *Digest Journal of Nanomaterials and Biostructures*, **8**, 845-854.

| | |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| Quantification of γ-Aminobutyric Acid in Sri Lankan Tea by Means of Ultra Performance Tandem Mass Spectrometry Elisabete Carvalho, P.A. Nimal Punyasiri, H.P.P. Sudarshana Somasiri, I. Sarath B. Abeysinghe and Stefan Martens | 525 |
| 5-(Hydroxymethyl)-2-furaldehyde Inhibits Adipogenic and Enhances Osteogenic Differentiation of Rat Bone Mesenchymal Stem Cells Xiang-ling Tan, Yan-Hong Zhang, Jian-Ping Cai, Li-Hua Zhu, Wen-Jie Ge and Xian Zhang | 529 |
| Variation of Glucosinolate Accumulation and Gene Expression of Transcription Factors at Different Stages of Chinese Cabbage Seedlings under Light and Dark Conditions Yeon Bok Kim, Jin-Hyuk Chun, Hye Ran Kim, Sun-Ju Kim, Yong Pyo Lim and Sang Un Park | 533 |
| Identification of the Hydroxamate Siderophore Ferricrocin in <i>Cladosporium cladosporioides</i> Nina Pourhassan, René Gagnon, Thomas Wichard and Jean-Philippe Bellenger | 539 |
| Structure Characterization and Adhesive Ability of a Polysaccharide from Tendrils of <i>Parthenocissus heterophylla</i> Li Zhang and Wenli Deng | 541 |
| Two Peptides, Cycloaspeptide A and Nazumamide A from a Sponge Associated Marine Actinobacterium <i>Salinispora</i> sp. Utpal Bose, Mark P. Hodson, P. Nicholas Shaw, John A. Fuerst and Amitha K. Hewavitharana | 545 |
| Full Assignments of the ^1H, ^{13}C and ^{15}N Magnetic Resonance Spectra of Two Porphyrin Compounds Qi-Feng Chen, Yao-Nan Wang, Ling Wang, Xi-Xian Jian, Dong-Lin Chen, Ming Zhao and Feng-Peng Wang | 547 |
| The Effects of <i>Salacia reticulata</i> on Anti-Cellular Oxidants and Melanogenesis Inhibition in α-MSH-stimulated and UV Irradiated B16 Melanoma Cells Prasit Suwannalert, Ryusho Kariya, Ikuko Suzu and Seiji Okada | 551 |
| Korean Propolis Suppresses Angiogenesis through Inhibition of Tube Formation and Endothelial Cell Proliferation Seon-Il Park, Toshiro Ohta, Shigenori Kumazawa, Mira Jun and Mok-Ryeon Ahn | 555 |
| ETAS, an Enzyme-treated Asparagus Extract, Attenuates Amyloid β-Induced Cellular Disorder in PC12 Cells Junetsu Ogasawara, Tomohiro Ito, Koji Wakame, Kentaro Kitadate, Takuya Sakurai, Shogo Sato, Yoshinaga Ishibashi, Tetsuya Izawa, Kazuto Takahashi, Hitoshi Ishida, Ichiro Takabatake, Takako Kizaki and Hideki Ohno | 561 |
| An Integrated Approach to the Evaluation of a Metabolomic Fingerprint for a Phytocomplex. Focus on Artichoke [<i>Cynara cardunculus</i> subsp. <i>scolymus</i>] Leaf Giada Fodaroni, Michela Burico, Anna Gaetano, Anna Maidecchi, Rita Pagiotti, Luisa Mattoli, Pietro Traldi and Eugenio Ragazzi | 565 |
| Cytotoxicity and Antimicrobial Activity of the Essential Oil from <i>Satureja montana</i> subsp. <i>pisidica</i> (Lamiaceae) Tatjana Kundaković, Tatjana Stanojković, Branka Kolundžija, Stevan Marković, Branka Šukilović, Marina Milenković and Branislava Lakušić | 569 |
| Chemical Composition of Essential Oils of <i>Grindelia squarrosa</i> and <i>G. hirsutula</i> Katalin Veres, Orsolya Roza, Eszter Laczkó-Zöld and Judit Hohmann | 573 |
| Seasonal Influence on the Essential Oil of <i>Eucalyptus microcorys</i> Flávia N. M. Oliveira, Gilmar A. C. Fortes, José R. Paula, Pedro H. Ferri and Suzana C. Santos | 575 |
| Composition of the Essential Oil of Wild Grown Caraway in Meadows of the Vienna Region (Austria) Remigius Chizzola | 581 |
| Volatile Compounds from Roots, Stems and Leaves of <i>Angelica acutiloba</i> growing in Taiwan Hsin-Chun Chen, Yi-Jr Tsai, Li-Yun Lin, Chin-Sheng Wu, Shan-Pao Tai, Yu-Chang Chen and Hsiu-Mei Chiang | 583 |
| Anti-oxidant, Anti-inflammatory and Anti-proliferative Activities of Moroccan Commercial Essential Oils Smail Aazza, Badiia Lyoussi, Cristina Megias, Isabel Cortés-Giraldo, Javier Vioque, A. Cristina Figueiredo and Maria G. Miguel | 587 |

Natural Product Communications

2014

Volume 9, Number 4

Contents

| <u>Original Paper</u> | <u>Page</u> |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| Activation of Cell-mediated Immunity by <i>Morinda citrifolia</i> Fruit Extract and Its Constituents Kazuya Murata, Yumi Abe, Megumi Futamura-Masuda, Akemi Uwaya, Fumiyuki Isami, and Hideaki Matsuda | 445 |
| A New Pyrrolsesquiterpene from the Terrestrial <i>Streptomyces</i> sp. Hd7-21 Dong-Ze Liu and Bo-Wen Liang | 451 |
| Structure-Activity Relationships of Tanshinones in Activating Nrf2. A DFT Study and Implications for Multifunctional Antioxidant Discovery You-Min Sun, Zheng-Tao Xiao and Hong-Yu Zhang | 453 |
| Chemical Modifications of Cinchona Alkaloids Lead to Enhanced Inhibition of Human Butyrylcholinesterase Daniela Karlsson, Adyary Fallarero, Pravin Shinde, Anju CP, Igor Busygin, Reko Leino, C. Gopi Mohan and Pia Vuorela | 455 |
| Alkaloids from Marine Sponges as Stimulators of Initial Stages of Development of Agricultural Plants Mikhail M. Anisimov, Elena L. Chaikina and Natalia K. Utkina | 459 |
| Crinane Alkaloids of the Amaryllidaceae with Cytotoxic Effects in Human Cervical Adenocarcinoma (HeLa) Cells Jerald J. Nair, Lucie Rárová, Miroslav Strnad, Jaume Bastida, Lee Cheesman and Johannes van Staden | 461 |
| Alkaloids from <i>Xylariaceae</i> sp., a Marine-derived Fungus Xu-Hua Nong, Xiao-Yong Zhang, Xin-Ya Xu, Yun-Lin Sun and Shu-Hua Qi | 467 |
| Occurrence of a Taurine Derivative in an Antarctic Glass Sponge Marianna Carbone, Laura Núñez-Pons, M. Letizia Ciavatta, Francesco Castelluccio, Conxita Avila and Margherita Gavagnin | 469 |
| Two New Thyminenol Derivatives from the Marine Sponge <i>Haliclona</i> sp. Bin Wang, Yaocai Lin, Yinning Chen and Riming Huang | 471 |
| Decorosides A and B, Cytotoxic Flavonoid Glycosides from the Leaves of <i>Rhododendron decorum</i> Mostafa E. Rateb, Hossam M. Hassan, El-Shaimaa A. Arafa, Marcel Jaspars and Rainer Ebel | 473 |
| In vitro Cultures of <i>Bituminaria bituminosa</i>: Pterocarpan, Furanocoumarin and Isoflavone Production and Cytotoxic Activity Evaluation Francesca D'Angiolillo, Laura Pistelli, Cecilia Noccioli, Barbara Ruffoni, Simona Piaggi, Roberto Scarpato and Luisa Pistelli | 477 |
| In Vitro Antioxidant Activity and Phenolic Content of <i>Cedrus brevifolia</i> Bark Elena Cretu, Juha-Pekka Salminen, Maarit Karonen, Anca Miron, Christiana Charalambous, Andreas I. Constantinou and Ana Clara Aprotosoai | 481 |
| Evaluation of Bioactive Components and Antioxidant and Anticancer Properties of Citrus Wastes Generated During Bioethanol Production Soon Jae Im, Jae-Hoon Kim and Min Young Kim | 483 |
| A New Coumarin and Cytotoxic Activities of Constituents from <i>Cinnamomum cassia</i> Tran Minh Ngoc, Nguyen Xuan Nhiem, Nguyen Minh Khoi, Doan Cao Son, Tran Viet Hung and Phan Van Kiem | 487 |
| Coumarin Compounds in <i>Coronilla scorpioides</i> Callus Cultures Anna Piovan, Raffaella Filippini and Gabriella Innocenti | 489 |
| A New Isocoumarin from <i>Cajanus cajan</i> (Fabaceae) Virginia F. Rodrigues, Rodrigo R. Oliveira and Maria Raquel G. Vega | 493 |
| Styryllactones and Acetogenins from the Fruits of <i>Goniothalamus macrocalyx</i> Quy Hung Trieu, Huong Doan Thi Mai, Van CuongPham, Marc Litaudon, Françoise Gueritte, Pascal Retailleau, Isabelle Schmitz-Afonso, Olinda Gimello, Van Hung Nguyen and Van Minh Chau | 495 |
| Potent Acetylcholinesterase Inhibitory Compounds from <i>Myristica fragrans</i> To Dao Cuong, Tran Manh Hung, Hyoung Yun Han, Hang Sik Roh, Ji-Hyeon Seok, Jong Kwon Lee, Ja Young Jeong, Jae Sue Choi, Jeong Ah Kim and Byung Sun Min | 499 |
| Clastogenic Effect of Atranorin, Evernic acid, and Usnic Acid on Human Lymphocytes Gordana S. Stojanović, Miroslava Stanković, Igor Ž. Stojanović, Ivan Palić, Vesna Milovanović and Sofija Rančić | 503 |
| MAO-A Inhibition Profiles of Some Benzophenone Glucosides from <i>Gentiana verna</i> subsp. <i>pontica</i> Duygu Kaya, Anna K. Jäger, Funda N. Yaşın and Tayfun Ersöz | 505 |
| Phytochemical Investigations of <i>Lonchocarpus</i> Bark Extracts from Monteverde, Costa Rica Caitlin E. Deskins, Bernhard Vogler, Noura S. Dosoky, Bhuwan K. Chhetri, William A. Haber and William N. Setzer | 507 |
| Immune Enhancing Effects of <i>Echinacea purpurea</i> Root Extract by Reducing Regulatory T Cell Number and Function Hyung-Ran Kim, Sei-Kwan Oh, Woosung Lim, Hyeon Kook Lee, Byung-In Moon and Ju Young Seoh | 511 |
| Isocorilagin, a Cholinesterase Inhibitor from <i>Phyllanthus niruri</i> Yee-Hui Koay, Alireza Basiri, Vikneswaran Murugaiyah and Kit-Lam Chan | 515 |
| Antioxidant Activity and Phenolic Content of <i>Bergenia crassifolia</i>, <i>B. x ornata</i> and <i>B. ciliata</i> Helena Hendrychová, Anna Vildová, Nina Kočevár-Glavač, Lenka Tůmová, Elnura Abdykerimova Kanybekovna and Jiří Tůma | 519 |
| Antioxidant Activity and Total Phenolic Contents of Three <i>Bupleurum</i> Taxa Hyeusoo Kim, Sea Hyun Kim and Kyeong Won Yun | 523 |

Continued inside backcover