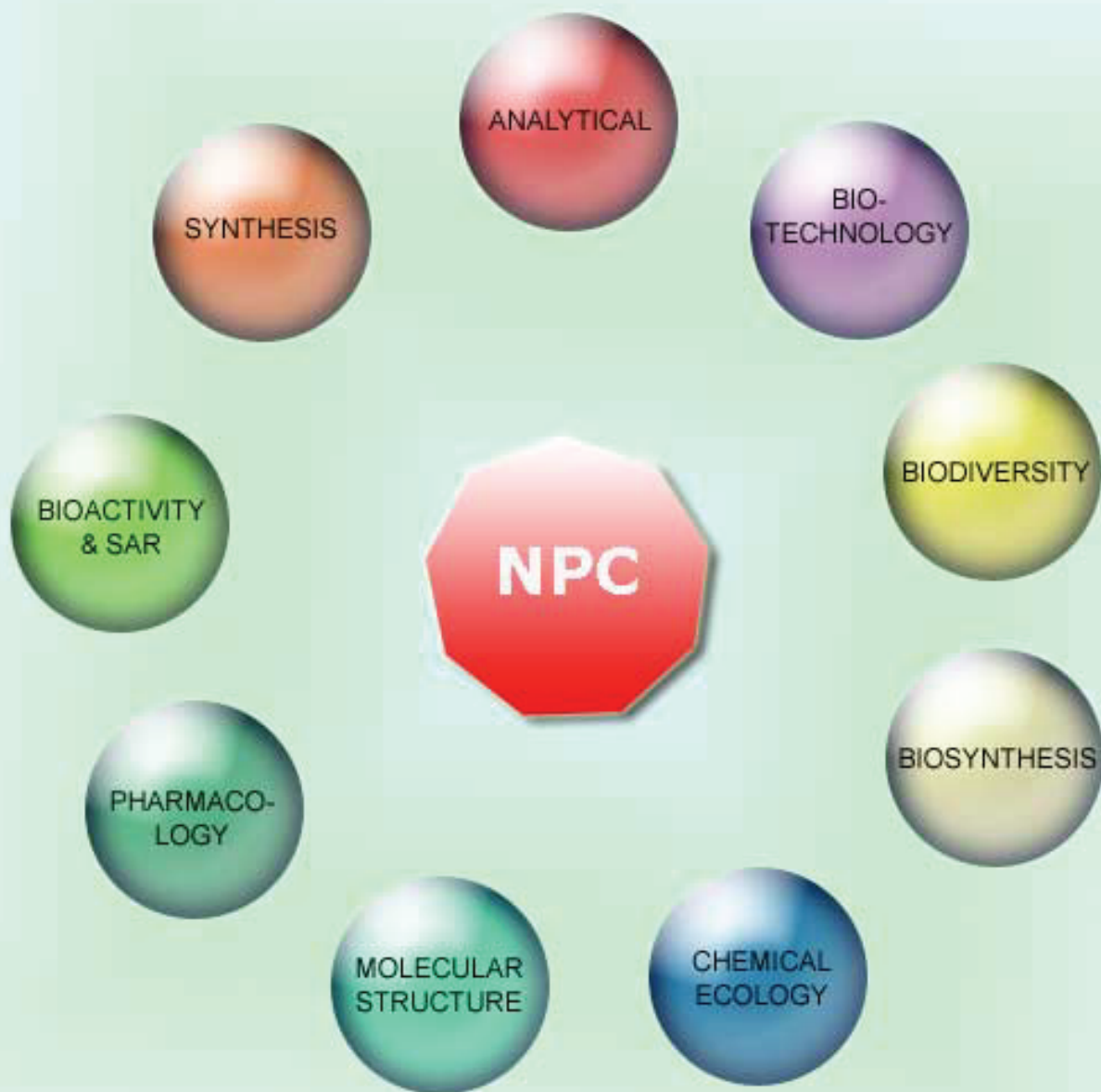


NATURAL PRODUCT COMMUNICATIONS

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



Volume 13. Issue 2. Pages 115-234. 2018
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 MAURIZIO BRUNO**

Department STEBICEF,
University of Palermo, Viale delle Scienze,
Parco d'Orleans II - 90128 Palermo, Italy
maurizio.bruno@unipa.it

PROFESSOR CARMEN MARTIN-CORDERO

Department of Pharmacology, Faculty of Pharmacy,
University of Seville, Seville, Spain
carmenmc@us.es

PROFESSOR VLADIMIR I. KALININ

G.B. Elyakov Pacific Institute of Bioorganic Chemistry,
Far Eastern Branch, Russian Academy of Sciences,
Pr. 100-letya Vladivostoka 159, 690022,
Vladivostok, Russian Federation
kalininv@piboc.dvo.ru

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 PING-JYUN SUNG

National Museum of Marine Biology and Aquarium
Checheng, Pingtung 944
Taiwan
pjsung@nmba.gov.tw

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

Institute of Pharmaceutical Science
Faculty of Life Sciences & Medicine
King's College London, Britannia House
7 Trinity Street, London SE1 1DB, UK
david.thurston@kcl.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. Giovanni Appendino
Novara, Italy

Prof. Norbert Arnold
Halle, Germany

Prof. Yoshinori Asakawa
Tokushima, Japan

Prof. Vassaya Bankova
Sofia, Bulgaria

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

Prof. Anna R. Bilia
Florence, Italy

Prof. Geoffrey Cordell
Chicago, IL, USA

Prof. Fatih Demirci
Eskişehir, Turkey

Prof. Francesco Epifano
Chieti Scalo, Italy

Prof. Ana Cristina Figueiredo
Lisbon, Portugal

Prof. Cristina Gracia-Viguera
Murcia, Spain

Dr. Christopher Gray
Saint John, NB, Canada

Prof. Dominique Guillaume
Reims, France

Prof. Duvvuru Gunasekar
Tirupati, India

Prof. Hisahiro Hagiwara
Niigata, Japan

Prof. Judith Hohmann
Szeged, Hungary

Prof. Tsukasa Iwashina
Tsukuba, Japan

Prof. Leopold Jirovetz
Vienna, Austria

Prof. Phan Van Kiem
Hanoi, Vietnam

Prof. Niel A. Koorbanally
Durban, South Africa

Prof. Chiaki Kuroda
Tokyo, Japan

Prof. Hartmut Laatsch
Gottingen, Germany

Prof. Marie Laccaille-Dubois
Dijon, France

Prof. Shoei-Sheng Lee
Taipei, Taiwan

Prof. M. Soledade C. Pedras
Saskatoon, Canada

Prof. Luc Pieters
Antwerp, Belgium

Prof. Peter Proksch
Düsseldorf, Germany

Prof. Phila Raharivelomanana
Tahiti, French Polynesia

Prof. Stefano Serra
Milano, Italy

Dr. Bikram Singh
Palampur, India

Prof. Marina Stefova
Skopje, Republic of Macedonia

Prof. Leandros A. Skaltsounis
Zografou, Greece

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. 2017 subscription price: US\$2,595 (Print, ISSN# 1934-578X); US\$2,595 (Web edition, ISSN# 1555-9475); US\$2,995 (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.

Cytotoxic Effect of Aeruginosin-865, Resveratrol and Capsaicin on Mouse Fibroblasts and Cells Derived from Fallow Deer

Ivana Veselá^{a*}, Petra Celá Kolísková^a, Vendula Kuchařová^a, Jaroslava Tomenendálová^a, Veronika Kováčková^b, Jiří Pikula^b, Barbora Repková^a, Polina Rapekta^a, Pavel Hrouzek^c, José Cheel^f and Jaroslav Doubek^a

^aDepartment of Physiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

^bDepartment of Ecology and Diseases of Game, Fish and Bees, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

^cCentre Algatech, Institute of Microbiology, The Czech Academy of Sciences (CAS) v.v.i., Trebon, Czech Republic

veselai@vfu.cz

Received: December 1st, 2017; Accepted: January 4th, 2018

Natural substances offer interesting bioactivity patterns including antiproliferative, antioxidant or cytotoxic effects. However, the safety profile of many of them has not been extensively determined. In this study, the cytotoxic effect of Aeruginosin-865, resveratrol and capsaicin at different concentrations was tested on normal mouse cells (NIH/3T3) and tumour fibroblasts (WEHI-13VAR) as well as on liver- and kidney-derived cells from fallow deer. A lactate dehydrogenase cytotoxicity assay kit was used to measure cell death in response to treatment with the test substances. It was found that NIH/3T3 cells tolerated Aeruginosin-865 (10–200 µM) and resveratrol (5–100 µM) treatment without any cytotoxic effect, while capsaicin exerted a cytotoxic effect only at the highest tested concentration (200 µM). Mouse fibrosarcoma cells were more sensitive to the cytotoxic effect of all three compounds where Aeruginosin-865 (100–200 µM) and resveratrol (50–100 µM) showed high-dose cytotoxicity and capsaicin showed low- and high-dose cytotoxicity (25 µM and 200 µM). The three tested compounds at the highest concentrations were found to be cytotoxic to both liver- and kidney-derived cells from fallow deer. Overall, the results indicate that the cytotoxic effects of the three tested natural substances on cells derived from fallow deer and mouse tumour fibroblasts differ significantly from those exerted on normal fibroblasts. The results demonstrate the potential of these natural compounds as therapeutic agents and pave the way for future *in vivo* toxicological investigations.

Keywords: Aeruginosin-865, Resveratrol, Capsaicin, Fibroblast, Fallow deer, Cell culture, Lactate dehydrogenase, Cytotoxicity.

Natural substances offer interesting biological properties such as antiproliferative, antioxidant or cytotoxic activities. However, their potential utilization as therapeutic agents requires determination of their safety profiles. In this research article, we focus on the effects of Aeruginosin-865, resveratrol and capsaicin. The aeruginosin family represents more than 500 aeruginosin variants that have been isolated from several cyanobacteria and marine sponges thus far [1, 2]. Cyanobacteria produce numerous secondary metabolites, which have many different functions, and several of them are cytotoxic [3]. On the other hand, a recently described class of linear peptides called aeruginosins exhibit varying degrees of inhibitory activity against serine proteases [2]. Nearly all of the aeruginosins are composed of four subunits: an N-terminal hydroxy or acidic group, a large hydrophobic amino acid, a 2- carboxyperhydroindole core and a C-terminal guanidine-containing group [4]. The most studied aeruginosin variants have been isolated from *Nodularia* or *Microcystis* strains [5, 6]. Aeruginosin-865, a tetrapeptide isolated for the first time from the terrestrial cyanobacterium *Nostoc*, has been shown to have anti-inflammatory effects mediated by inhibition of the NF- κB signalling pathway [7], which subsequently lead to inhibition of transcription of genes playing a role in cell survival or inflammation progression. However, the exact mechanism underlying the anti-inflammatory effect of Aeruginosin-865 has not yet been fully elucidated.

Capsaicin (*trans*-8-methyl-N-vanillyl-6-nonenamide), an alkaloid found in the fruit of the *Capsicum* plant family, is the main molecule responsible for the typical pungency of these plants. Therefore, it represents a natural defence mechanism against herbivores and fungi. The effect of capsaicin on animals and humans has been studied for more than a century, yielding

promising results mainly in pain relief, inflammation, obesity and even cancer treatment or cancer prevention [8]. The anticancer property of capsaicin was tested on more than 80 different cell lines, predominantly of human origin. Most of the published studies agree that capsaicin shows an inhibitory effect on cancer cells, whereas “normal” cells tolerate capsaicin treatment without any effect on their viability and growth. The mechanism by which capsaicin provides an anticancer effect is still not fully elucidated, and there are probably additional modes of action. In fact, many authors have addressed cell-cycle arrest, inhibition of cell growth and proliferation or apoptosis induction [9, 10] as the possible mechanisms underlying the anticancer effect. Capsaicin has also been shown to have an anti-inflammatory effect similar to that of aeruginosine-865 [11].

Resveratrol is a polyphenol compound synthesized by various plant species such as grapevine, cranberries, broccoli or garlic, and the mechanisms by which it can prevent, arrest or delay tumour development have been elucidated [12,13]. As in the case of capsaicin, the mechanism of resveratrol action is still not satisfactorily explained. However, the disruption of mitochondrial transmembrane potential, increase in production of oxygen radicals or increase in the intracellular calcium concentration [13-15] may play essential roles in the benefits exhibited by these two compounds.

In the present study, the cytotoxic effects of Aeruginosin-865, resveratrol and capsaicin were investigated on two mouse cell lines: normal fibroblasts and tumour-transformed fibroblasts, which are a suitable animal model of carcinogenesis. Due to the increasing incidence of cancer in humans and animals, the results obtained could contribute to the use of these natural substances as potential

therapeutics in both human and veterinary medicine. Moreover, the effects of these compounds were tested on liver- and kidney-derived cells from fallow deer. We focused primarily on the effect of aeruginosin produced by cyanobacteria, because their intensive growth in freshwater supplies and terrestrial soil can lead to water and plant contamination, so grazers such as fallow deer are at the greatest risk [16]. Therefore, examination of the compounds produced by cyanobacteria is at the forefront of recent research. This study compares the cytotoxic effects obtained using a standard experimental model and a wildlife cell model.

We hypothesized that cells of different origins would show a variable response to the adverse effects of Aeruginosin-865, resveratrol and capsaicin, i.e. substances with potential medical applications. We tested this by using a range of concentrations and assaying cytotoxicity through lactate dehydrogenase activity (LDH).

The cytotoxicity of Aeruginosin-865, capsaicin and resveratrol to mouse fibroblasts is shown in Figure 1. 3T3 mouse fibroblast cells tolerated Aeruginosin-865 and resveratrol treatment without any cytotoxic effects, while capsaicin showed cytotoxicity only at the highest concentration ($p < 0.05$). Mouse fibrosarcoma cells were more sensitive to the cytotoxic effects of all three compounds. Aeruginosin-865 and resveratrol showed high-dose cytotoxicity. Statistical significance ($p < 0.05$) was only observed in the case of Aeruginosin-865, while the effect of resveratrol was nonsignificant ($p > 0.05$). Cancer cells were more responsive to the cytotoxic effect of capsaicin when compared with Aeruginosin-865 or resveratrol treatment. We detected low- and high-dose cytotoxicity on fibrosarcoma cells when treated with capsaicin with the level of significance $p < 0.05$.

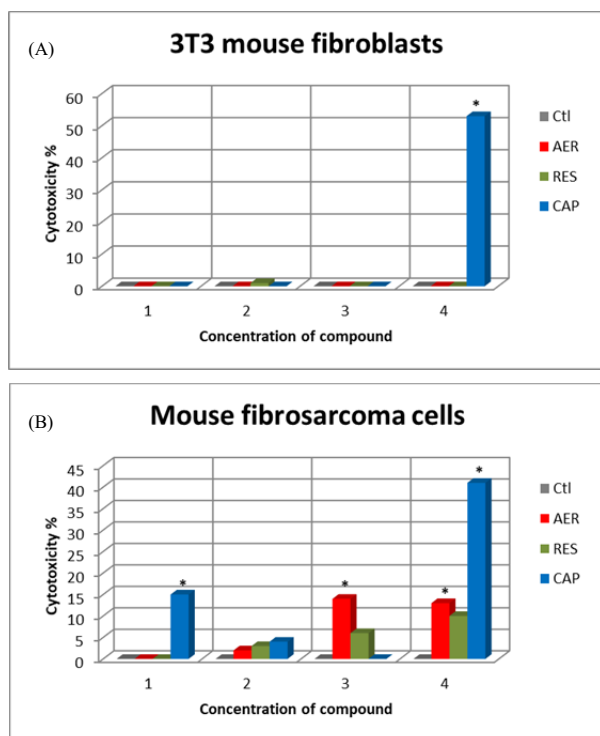


Figure 1: Cytotoxicity of Aeruginosin-865, resveratrol and capsaicin on 3T3 mouse fibroblasts (A) and mouse fibrosarcoma cells (B). Cells were treated with the test compounds at different concentrations for 24 hours. Group 1 – Aeruginosin-865 10 μ M, resveratrol 5 μ M, capsaicin 25 μ M. Group 2 – Aeruginosin-865 50 μ M, resveratrol 10 μ M, capsaicin 50 μ M. Group 3 – Aeruginosin-865 100 μ M, resveratrol 50 μ M, capsaicin 100 μ M. Group 4 – Aeruginosin-865 200 μ M, resveratrol 100 μ M, capsaicin 200 μ M. Controls represent untreated cells incubated with 0.1%–2% DMSO. * = $p < 0.05$ when compared with the control group.

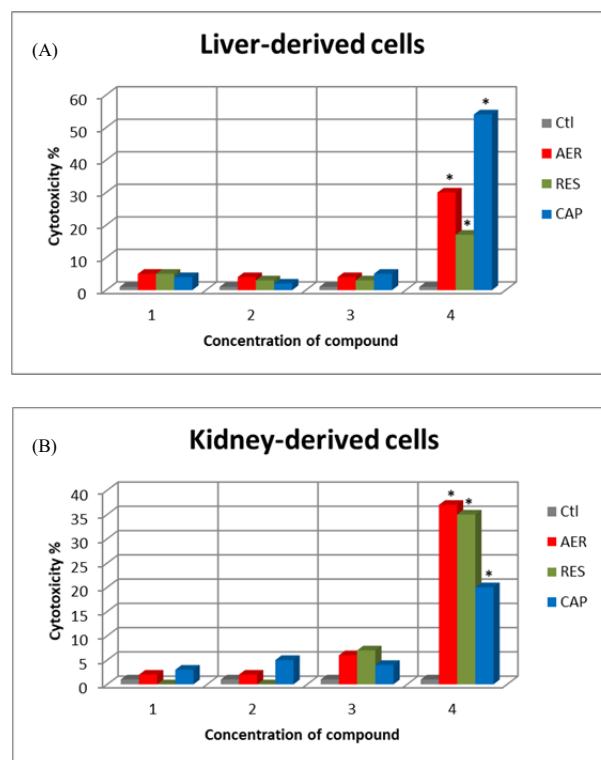


Figure 2: Cytotoxicity of Aeruginosin-865, resveratrol and capsaicin for fallow deer liver-derived cells (A) and kidney-derived cells (B). Cells were treated with test compounds at different concentrations for 24 hours. Group 1 – Aeruginosin-865 10 μ M, resveratrol 5 μ M, capsaicin 25 μ M. Group 2 – Aeruginosin-865 50 μ M, resveratrol 10 μ M, capsaicin 50 μ M. Group 3 – Aeruginosin-865 100 μ M, resveratrol 50 μ M, capsaicin 100 μ M. Group 4 – Aeruginosin-865 200 μ M, resveratrol 100 μ M, capsaicin 200 μ M. Controls represent untreated cells incubated with 0.1%–2% DMSO. * = $p < 0.05$ when compared with the control group.

The cytotoxicity of Aeruginosin-865, capsaicin and resveratrol to liver- and kidney-derived cells from the fallow deer is shown in Figure 2. The three compounds exerted a similar profile of cytotoxic effects in both tested cell lines. Aeruginosin-865, capsaicin and resveratrol did not show any significant effect at low concentrations but were found to exhibit a statistically significant cytotoxic effect ($p < 0.05$) on both liver- and kidney-derived cells. Liver-derived cells were twice as sensitive to capsaicin as kidney-derived cells. A similar effect was observed for resveratrol, but in this case kidney-derived cells were twice as sensitive as liver-derived cells. The cytotoxic effects of Aeruginosin-865 in liver- and kidney-derived cells were almost identical.

This work utilised an *in vitro* animal model of carcinogenesis to evaluate the cytotoxic effects of three different natural substances and simultaneously compared an *in vitro* mouse experimental model and a wildlife model represented by cells derived from fallow deer. According to Kapuścik *et al.* [7] Aeruginosin-865 did not show any cytotoxic effect in human lung microvascular endothelial cells (HLMVECs). A similar outcome was also confirmed by Faltermann *et al.* [3] for Aeruginosin-828A from *Planktothrix* strains in Huh7 human hepatoma cells. Furthermore, acute toxicity of Aeruginosin-828A to the crustacean *Thamnocephalus platyurus* has been documented [17, 18], but this effect has not been confirmed in zebrafish embryos or zebrafish liver organ cultures [3]. The current study presents for the first time the effect of Aeruginosin-865 on NIH/3T3 mouse normal fibroblasts and WEHI-13VAR mouse tumour fibroblasts. Aeruginosin-865 showed no cytotoxic effect on normal mouse fibroblasts, even at the highest concentration used in the test, in agreement with other studies [3, 7]. Concurrently, tumour-transformed mouse fibroblasts were sensitive to

Aeruginosin-865, mainly at the highest concentrations. These findings identify Aeruginosin-865 as a potential therapeutic agent for use in the prevention of cancer or as part of a combined therapy with radiation and chemotherapeutics. However, additional research is needed to better understand the exact mechanism of action and biological behaviour of Aeruginosin-865 in cancer cells. As the majority of aeruginosin variants are produced by aquatic cyanobacteria, it is understandable that environmental toxicity has been studied in sensitive freshwater organisms such as the crustacean *Thamnocephalus platyurus* [17, 18] or zebrafish [3]. Aeruginosin-865 is a novel class of aeruginosin variant that was discovered in a strain of soil cyanobacterium (*Nostoc* sp.) [7]. The lack of cell toxicity and anti-inflammatory properties of Aeruginosin-865 [7] encouraged the investigation of this compound in fallow deer-derived cells. Considering that the liver and kidney represent metabolically active organs and the pharmacodynamics of Aeruginosin-865 is not fully understood, we performed experiments on liver- and kidney-derived cells. Cytotoxicity of Aeruginosin-865 was found only at the highest used concentration (200 μM) in both cell types. On the other hand, toxicity of Aeruginosin 828A was reported for the crustacean *Thamnocephalus platyurus*, with lethal doses starting at 22.4 and 34.5 μM , respectively [17, 18]. Based on the cytotoxicity observed in liver- and kidney-derived cells, it may be hypothesized that possible structural modifications in aeruginosin variants might act as a factor contributing to the cytotoxic effect. Furthermore, freshwater organisms might be more sensitive to such compounds than terrestrial species.

Bley *et al.* [14] summarized the results of studies describing apoptotic or growth inhibitory effects of capsaicin which were selective for cancerous cells and left normal or noncancerous cells unharmed. Another study revealed variable sensitivity of different cell lines including human dermal fibroblasts (HDF) and mouse embryonic fibroblasts (NIH/3T3) and some cancerous cell lines. Capsaicin had no effect on the viability of dermal fibroblasts, whereas mouse embryonic fibroblasts were sensitive to capsaicin treatment, with the first cytotoxic effect observed at a dose of 50 μM and an IC_{50} around 200 μM . Among the cancerous cell lines, human breast carcinoma cells (MCF7) were the most sensitive, already displaying a cytotoxic effect at the lowest concentration (5 μM) [19]. Ghosh and Basu [20] compared the pro-apoptotic effects of capsaicin on fibrosarcoma cells (Meth A, CMS5) and mouse embryonic fibroblasts (MEFS). The authors reported that capsaicin treatment induced apoptosis only in fibrosarcoma cells with increasing reactive oxygen species (ROS) production. In the present study, normal fibroblasts showed a low sensitivity to capsaicin, but cancerous cells were sensitive to capsaicin even at a low concentration. This discontinuity between low- and high-concentration cytotoxicity of capsaicin may suggest that mouse fibrosarcoma cells respond to capsaicin exposure using a different mechanism when compared with normal fibroblasts. Isolated rat hepatocytes treated with capsaicin showed a cytotoxic effect only at a high concentration (LD_{50} 400 μM), whereas hepatoma cells (Hep G2) were eight times more sensitive (IC_{50} 50 μM) [21]. Similar results using a normal hepatic cell line (L-02) and a human hepatoma cancer cell line (SMMC-7721) were documented [22]. Normal hepatocytes showed a very slight decrease in viability (approximately 90%) at 300 μM of capsaicin whereas the viability of cancerous cells was markedly decreased (approximately 20%). The impact of capsaicin treatment on kidney cells has not been well studied. Cochereau *et al.* [23] described the cytotoxic effect of capsaicin in monkey kidney cells (Vero cells) when capsaicin at a concentration of 68 μM reduced the cell number by half compared with the control. In the current study, kidney-derived cells from fallow deer were, in contrast, more resistant to the cytotoxic effect

of a high concentration of capsaicin when compared with liver-derived cells. Further studies are needed to detect a possible toxic effect of capsaicin in different cells from different species and to clarify the mechanisms of its action.

Resveratrol was shown to inhibit growth and proliferation in many cancer cell lines with limited cytotoxicity toward normal cells [13, 15]. Other related compounds, oxyresveratrol or stilbene-based resveratrol analogues, are also considered to have antiproliferative or anticancer properties [24, 25]. Resveratrol decreased cell viability and induced apoptosis in HT1080 fibrosarcoma cells [26-28]. The effect on the cell viability was dose-dependent, showing a 50% decrease in the viable cell count at a dose of 50 μM [26, 28]. In our study we confirmed the dose-dependent inhibitory effect of resveratrol on mouse fibrosarcoma cells, but without any significant result at any concentration tested. In normal mouse fibroblasts resveratrol did not induce any cytotoxic effects. In liver- and kidney-derived cells from fallow deer, the highest concentration of resveratrol significantly increased cytotoxicity, leading to reduction of cell viability.

Our findings indicate that Aeruginosin-865, resveratrol and capsaicin differ significantly in their cytotoxic effect on cells derived from fallow deer; moreover, the dissimilar mechanism of action is observed in mouse fibrosarcoma cells in comparison with normal mouse fibroblasts. Further studies are necessary to clarify and better understand the effect of these natural substances on normal and/or tumour cells in different animal species.

Experimental

Cell cultures: The commercially available mouse normal cell line NIH/3T3 (in the collection of the Department of Physiology) and the tumour fibroblast cell line WEHI-13VAR (ATCC® CRL-2148™) were tested. Cell lines were cultured in MEM Alpha Medium (Thermo Fisher Scientific, Waltham, MA, USA) for normal cells and RPMI-1640 medium (Sigma-Aldrich, St. Louis, Missouri, USA) for tumour cells. Liver- and kidney-derived cells from fallow deer were obtained as previously described [29] and cultured in DMEM/F12 medium (Biosera, Boussens, France). Each medium was supplemented with 10% FBS (Sigma-Aldrich, St. Louis, Missouri, USA) and 1% Penicillin-Streptomycin (Sigma-Aldrich, St. Louis, Missouri, USA). Cells were cultured in 96-well plates in the appropriate complete medium and placed in a 5% CO_2 incubator at 37°C.

Natural substances: Aeruginosin-865 was provided by the Laboratory of Algal Biotechnology, Institute of Microbiology, Czech Academy of Sciences in Trebon, where it was obtained according to a previously described isolation procedure [7]. Resveratrol (554325) and capsaicin (M2028) were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). These substances were applied in parallel to all types of cells at different concentrations and incubated for 24 hours. DMSO (D4540, Sigma-Aldrich, St. Louis, Missouri, USA) was used to dissolve the natural substances in the concentration range of 0.1%–2% to prepare a stock solution. Exposure solutions were prepared immediately prior to the experiment. We used Aeruginosin-865 in 10 μM , 50 μM , 100 μM and 200 μM concentrations, resveratrol in 5 μM , 10 μM , 50 μM and 100 μM concentrations and capsaicin in 25 μM , 50 μM , 100 μM and 200 μM concentrations.

Treatment schedule for cytotoxicity and LDH test: To evaluate the cytotoxicity, we used LDH cytotoxicity assay kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. At first, an optimal cell concentration was tested in a

preliminary experiment. Then the cells were seeded in 96-well plate at a density of 2×10^4 cells/well in the appropriate medium and incubated overnight. On the second day, test solutions of Aeruginosin-865, resveratrol and capsaicin at different concentrations were applied, and the cells were further cultured for 24 hours at 37°C in a 5% CO₂ atmosphere. The next day, the reaction mixture was added to each sample. The reaction was performed for 30 min at 37°C protected from light and then stopped by the addition of Stop Solution. The absorbance was read at 490 nm and 680 nm on a SynergyHT (BioTek, USA) instrument.

LDH released from the cytosol of damaged cells induces tetrazolium conversion to a red formazan of intensity proportional to the amount of LDH released. A positive control (maximum LDH activity) was established by treatment with Lysis Buffer (10X)

provided by the kit, and a negative control (spontaneous LDH activity) was established by treatment with sterile distilled water. To exclude a potential cytotoxic effect of the solvent, we performed a solvent control reaction with 0.1%–2 % DMSO on untreated cells.

Statistics: The statistical significance of the difference between each concentration vs the control treated sample was evaluated using MedCalc statistical software. We applied T-test for statistical analysis. Differences with a $p < 0.05$ were considered statistically significant.

Acknowledgments - The authors thank IAPG AS CR, v. v. i. for the loan of the SynergyHT reader. This work was supported by the Internal Grant Agency of the University of Veterinary and Pharmaceutical Sciences Brno, Grant No. 109/2016/FVL.

References

- [1] Welker M, Marsálek B, Sejnohová L, von Döhren H. (2004) Detection and identification of oligopeptides in *Microcystis* (cyanobacteria) colonies: toward an understanding of metabolic diversity. *Peptides*, **27**, 2090-2103.
- [2] Ersmark K, Del Valle JR, Hanessian S. (2008) Chemistry and biology of the aeruginosin family of serine protease inhibitors. *Angewandte Chemie International Edition*, **47**, 1202-1223.
- [3] Faltermann S, Hutter S, Christen V, Hettich T, Fent K. (2016) Anti-inflammatory activity of cyanobacterial serine protease inhibitors Aeruginosin 828A and Cyanopeptolin 1020 in human hepatoma cell line Huh7 and effects in zebrafish (*Danio rerio*). *Toxins*, **8**, 219-233.
- [4] Ishida K, Welker M, Christiansen G, Cadel-Six S, Bouchier C, Dittmann E, Hertweck C, Tandeau de Marsac N. (2009) Plasticity and evolution of aeruginosin biosynthesis in cyanobacteria. *Applied and Environmental Microbiology*, **75**, 2017-2026.
- [5] Elkobi-Peer S, Singh RK, Mohapatra TM, Tiwari SP, Carmeli S. (2013) Aeruginosins from a *Microcystis* sp. bloom material collected in Varanasi, India. *Journal of Natural Products*, **76**, 1187-1190.
- [6] Fewer DP, Jokela J, Paukku E, Österholm J, Wahlsten M, Permi P, Aitio O, Rouhiainen L, Gomez-Saez GV, Sivonen K. (2013) New structural variants of aeruginosin produced by the toxic bloom forming cyanobacterium *Nodularia spumigena*. *PLoS One*, **8**, e73618.
- [7] Kapuščík A, Hrouzek P, Kuzma M, Bártošová S, Novák P, Jokela J, Pflüger M, Eger A, Hundsberger H, Kopecký J. (2013) Novel Aeruginosin-865 from *Nostoc* sp. as a potent anti-inflammatory agent. *Chembiochem*, **14**, 2329-2337.
- [8] Sharma SK, Vij AS, Sharma M. (2013) Mechanisms and clinical uses of capsaicin. *European Journal of Pharmacology*, **720**, 55-62.
- [9] Clark R, Lee SH. (2016) Anticancer properties of capsaicin against human cancer. *Anticancer Research*, **36**, 837-843.
- [10] Chapa-Oliver AM, Mejia-Teniente L. (2016) Capsaicin: from plants to a cancer-suppressing Agent. *Molecules*, **21**, 931.
- [11] Reyes-Escogido Mde L, Gonzalez-Mondragon EG, Vazquez-Tzompantzi E. (2011) Chemical and pharmacological aspects of capsaicin. *Molecules*, **16**, 1253-1270.
- [12] Kim MY, Trudel LJ, Wogan GN. (2009) Apoptosis induced by capsaicin and resveratrol in colon carcinoma cells requires nitric oxide production and caspase activation. *Anticancer Research*, **29**, 3733-3740.
- [13] Ferraz da Costa DC, Fialho E, Silva JL. (2017) Cancer chemoprevention by resveratrol: the p53 tumor suppressor protein as a promising molecular target. *Molecules*, **22**, 1014.
- [14] Bley K, Boorman G, Mohammad B, McKenzie D, Babbar S. (2012) A comprehensive review of the carcinogenic and anticarcinogenic potential of capsaicin. *Toxicologic Pathology*, **40**, 847-873.
- [15] Varoni EM, Lo Faro AF, Sharifi-Rad J, Iriti M. (2016) Anticancer molecular mechanisms of Resveratrol. *Frontiers in Nutrition*, **3**, 8.
- [16] Blom JF, Robinson JA, Jüttner F. (2001) High grazer toxicity of [D-Asp(3),(E)-Dhb(7)]microcystin-RR of *Planktothrix rubescens* as compared to different microcystins. *Toxicon*, **39**, 1923-1932.
- [17] Kohler E, Grundler V, Häussinger D, Kurmayer R, Gademann K, Pernthaler J, Blom JF. (2014) The toxicity and enzyme activity of a chlorine and sulfate containing aeruginosin isolated from a non-microcystin-producing *Planktothrix* strain. *Harmful Algae*, **39**, 154-160.
- [18] Scherer M, Bezold D, Gademann K. (2016) Investigating the toxicity of the aeruginosin chlorosulfopeptides by chemical synthesis. *Angewandte Chemie International Edition in English*, **55**, 9427-9431.
- [19] Lewinska A, Chochrek P, Smolag K, Rawksa E, Wnuk M. (2015) Oxidant-based anticancer activity of a novel synthetic analogue of capsaicin, capsaicin epoxide. *Redox Report*, **20**, 116-125.
- [20] Ghosh AK, Basu S. (2010) Fas-associated factor 1 is a negative regulator in capsaicin induced cancer cell apoptosis. *Cancer Letters*, **287**, 142-149.
- [21] Galati G, O'Brien PJ. (2003) Cytoprotective and anticancer properties of coenzyme Q versus capsaicin. *Biofactors*, **18**, 195-205.
- [22] Bu HQ, Cai K, Shen F, Bao XD, Xu Y, Yu F, Pan HQ, Chen CH, Du ZJ, Cui JH. (2015) Induction of apoptosis by capsaicin in hepatocellular cancer cell line SMMC-7721 is mediated through ROS generation and activation of JNK and p38 MAPK pathways. *Neoplasia*, **62**, 582-591.
- [23] Cochereau C, Sanchez D, Creppy EE. (1997) Tyrosine prevents capsaicin-induced protein synthesis inhibition in cultured cells. *Toxicology*, **117**, 133-139.
- [24] Chillemi R, Sciuto S, Spatafora C, Tringali C. (2007) Anti-tumor properties of stilbene-based resveratrol analogues: Recent results. *Natural Product Communications*, **2**, 499-513.
- [25] Sintuyanon N, Phoolcharoen W, Pavasant P, Soompon S. (2017) Resveratrol demonstrated higher antiproliferative and antiangiogenic efficacy compared with oxyresveratrol on head and neck squamous cell carcinoma cell lines. *Natural Product Communications*, **12**, 1781-1784.
- [26] Gweon EJ, Kim SJ. (2013) Resveratrol induces MMP-9 and cell migration via the p38 kinase and PI-3K pathways in HT1080 human fibrosarcoma cells. *Oncology Reports*, **29**, 826-834.
- [27] Harati K, Slodnik P, Chromik AM, Goertz O, Hirsch T, Kapalschinski N, Klein-Hitpass L, Kolbensschlag J, Uhl W, Lehnhardt M, Daigeler A. (2015) Resveratrol induces apoptosis and alters gene expression in human fibrosarcoma cells. *Anticancer Research*, **35**, 767-774.
- [28] Lee SJ, Kim MM. (2011) Resveratrol with antioxidant activity inhibits matrix metalloproteinase via modulation of SIRT1 in human fibrosarcoma cells. *Life Sciences*, **88**, 465-472.
- [29] Kovacova V, Abdelsalam EE, Bandouchova H, Brichta J, Havelkova B, Piacck V, Vitula F, Pikula J. (2016) Cytotoxicity of ketamine, xylazine and Hellabrunn mixture in liver-, heart- and kidney-derived cells from fallow deer. *Neuroendocrinology Letters*, **37**, 78-83.

Green Soybean Extract Ameliorates Dextran Sodium Sulfate-Induced Colitis Yuko Yoshikawa, Takuya Murakami, Yuki Katayanagi, Kensuke Yasui, Yasushi Ohgo, Shinjiro Imai and Norio Ohashi	209
Chemical Composition and Variability of Leaf and Berry oils from Corsican <i>Juniperus macrocarpa</i> Joséphine Ottavioli, Ange Bighelli, Joseph Casanova and Félix Tomi	213
Composition and Chemical Variability of Essential Oils Isolated from Aerial Parts of <i>Cassipouita fliformis</i> from Côte d'Ivoire Zana A. Ouattara, Nouho Sangaré, A. Janat Mamyrbekova-Bekro, Yves-Alain Békro, Pierre Tomi, Mathieu Paoli, Ange Bighelli and Felix Tomi	217
Chemical Composition of the Essential Oil from the Roots of <i>Ferula kuhistanica</i> Growing Wild in Tajikistan Payrav D. Khalifaev, Farukh S. Sharopov, Abduahad Safomuddin, Sodik Numonov, Mahinur Bakri, Maidina Habasi, Haji Akber Aisa and William N. Setzer	219
Chemical Composition, Antimicrobial and Anti-inflammatory Activity of Algerian <i>Juniperus phoenicea</i> Essential Oils Wafae Abdelli, Fouad Bahri, Martina Höferl, Juergen Wanner, Erich Schmidt and Leopold Jirovetz	223
Chemical Composition, Antioxidant, Antimicrobial, and α-Glucosidase Activities of Essential Oils of <i>Hornstedtia scyphifera</i> (Zingiberaceae) Siti Ernieyanti Hashim and Hasnah Mohd Sirat	229

Additions/Corrections

Methyl 3-(5-(prop-1-yn-1-yl)thiophen-2-yl)propanoate: A Rare Acetylene Derivative from <i>Artemisia absinthium</i> Root Essential Oil Polina D. Blagojević, Marko S. Pešić and Niko S. Radulović <i>Natural Product Communications</i> (2017) 12 (4), 603-606	233
--	-----

<u>Manuscripts in Press</u>	234
------------------------------------	-----

Natural Product Communications

2018

Volume 13, Number 2

Contents

<u>Original Paper</u>	<u>Page</u>
Antiviral Activity of the Sesquiterpene Lactones from <i>Centipeda minima</i> against Influenza A Virus <i>in vitro</i> Xiaoli Zhang, Jun He, Weihuan Huang, Huibin Huang, Zeming Zhang, Jiajian Wang, Li Yang, Guocai Wang, Yifei Wang and Yaolan Li	115
A New Diepoxy abietaneolide from <i>Suregada multiflora</i> Humaira Yasmeen Gondal, Muhammad Nisar and M. Iqbal Choudhary	121
A New Cembrane, from Soft Coral Genus <i>Sarcophyton</i> in Borneo Takashi Kamada, Intan Irma Zaniil, Chin-Soon Phan and Charles Santharaju Vairappan	123
Novel <i>Ent</i>-Kaurene Glycosides with Eight Glycosyl Units from <i>Stevia rebaudiana</i> Indra Prakash, Bin Wang, Gil Ma, George Harrigan, Steven F. Sukits, Krishna P. Devkota, Romila D. Charan, Ryan Donovan and Tara M. Snyder	125
Chemical Constituents of <i>Vitex trifolia</i> Leaves Ninh Khac Ban, Nguyen Thi Kim Thoa, Tran My Linh, Vu Huong Giang, Do Thi Trang, Nguyen Xuan Nhiem, Bui Huu Tai, Tran Hong Quang, Pham Hai Yen, Chau Van Minh and Phan Van Kiem	129
Oleanane-type Triterpenes with Highly-Substituted Oxygen Functional Groups from the Flower Buds of <i>Camellia sinensis</i> and Their Inhibitory Effects against NO Production and HSV-1 Taichi Yoneda, Seikou Nakamura, Keiko Ogawa, Tomoko Matsumoto, Souichi Nakashima, Kiriko Matsumura, Aoi Tanaka, Kaori Ryu, Masashi Fukaya, Masahiro Fujimuro, Masayuki Yoshikawa and Hisashi Matsuda	131
Triterpene Glycosides from the Sea Cucumber <i>Eupentacta fraudatrix</i>. Structure and Cytotoxic action of Cucumarioside D with a Terminal 3-O-Me-Glucose Residue Unique for this Species Alexandra S. Silchenko, Anatoly I. Kalinovsky, Sergey A. Avilov, Roman S. Popov, Vladimir I Kalinin, Pelageya V. Andrijaschenko, Pavel S. Dmitrenok and Ekaterina A. Yurchenko	137
Methylobamine, a UVA-Absorbing Compound from the Plant-Associated Bacteria <i>Methylobacterium</i> sp. Tsunashi Kamo, Syuntaro Hiradate, Ken Suzuki, Ichiro Fujita, Shinji Yamaki, Tadashi Yoneda, Motoo Koitabashi and Shigenobu Yoshida	141
Preparation and Regeneration of Protoplasts from the Ethyl Vincamine Producing Fungus CH1 (<i>Geomyces</i> sp.) Na Ren, Jiajia Liu, Dongliang Yang, Xiong Liu, Jing Zhou and Yingzi Peng	145
Two New Compounds from Medicinal Insect <i>Blaps japonensis</i> and Their Biological Evaluation Tao Zheng, Yan-Yong Ming, Zheng-Chao Tu, Fu Rong Xu and Yong-Xian Cheng	149
Isolation and Structure Determination of a New Lumichrome Glycoside Isolated from a Soil <i>Streptomyces</i> sp. KCB16C001 Sangkeun Son, Eun Kim, Jong Won Kim, Sung-Kyun Ko, Byeongsan Lee, Jung-Sook Lee, Young-Soo Hong, Jae-Hyuk Jang and Jong Seog Ahn	153
Antimicrobial Activity of the Constituents of <i>Dalbergia tonkinensis</i> and Structural-Bioactive Highlights Ninh The Son, Masataka Oda, Naoki Hayashi, Daiki Yamaguchi, Yu Kawagishi, Fumi Takahashi, Kenichi Harada, Nguyen Manh Cuong and Yoshiyasu Fukuyama	157
Chemical Composition and Cytotoxicity of <i>Kalanchoe pinnata</i> Leaves Extracts prepared using Accelerated System Extraction (ASE) Kassia M. F. Pereira, Simone S. Grecco, Carlos R. Figueiredo, Jorge K. Hosomi, Mari U. Nakamura and João Henrique G. Lago	163
Development of a Bioproduct for Medicinal Use with Extracts of <i>Zuccagnia</i>-type Propolis Ana Salas, Iris Catiana Zampini, Luis Maldonado and María Inés Isla	167
Argentinean <i>Larrea</i> Dry Extracts with Potential Use in Vaginal Candidiasis María Alejandra Moreno, Susana Córdoba, Iris Catiana Zampini, María Inés Mercado, Graciela Ponessa, Jorge Esteban Sayago, Liudis Leidy Pino Ramos, Guillermo Schmeda-Hirschmann and María Inés Isla	171
The Mechanisms of Shcisandrol A in Immune Function Modulation in Immunosuppressed Mice Guangyu Xu, Xu Liu, Chunmei Wang, He Li, Chengyi Zhang, Jianguang Chen and Jinghui Sun	175
Synthesis of Polyhydroxylated Aminonaphthazarins Related to Natural Pigments Galina I. Melman, Natalia D. Pokhilo, Lyubov N. Atopkina, Vladimir A. Denisenko and Victor Ph. Anufriev	181
A New Tannin from Fruits of <i>Torreya nucifera</i> with Protein Tyrosine Phosphatase 1B Inhibitory Activity Dao-Li Guo	185
Comprehensive Metabolomics Study of Traditionally Important <i>Rumex</i> Species Found in Western Himalayan Region Ritika Sharma, Rupali Jandrotia, Bikram Singh, Upendra Sharma and Dinesh Kumar	189
Antidiabetic Effects of the <i>Auricularia auricular</i> Polysaccharides Simulated Hydrolysates in Experimental Type-2 Diabetic Rats Aoxue Lu, Meng Shen, Zhiyu Fang, Yaoyao Xu, Mengen Yu, Shuang Wang, Yongjun Zhang and Weimin Wang	195
Cinobufacini from the Skin of <i>Bufo bufo gargarizans</i> Induces Apoptosis, Possibly via Activation of the Wnt/β-Catenin Pathway, in Human Osteosarcoma Cells Xiu-cai Ma, Hui-qiang Ding, Jian-dang Shi, Long Hei, Ning-kui Niu, Zhi-gang Suo, Yan-bing Shang, Song Lin, Fei-fei Pu and Zeng-wu Shao	201
Cytotoxic Effect of Aeruginosin-865, Resveratrol and Capsaicin on Mouse Fibroblasts and Cells Derived from Fallow Deer Ivana Veselá, Petra Celá Kolísková, Vendula Kuchařová, Jaroslava Tomenendálová, Veronika Kováčková, Jiří Pikula, Barbora Repková, Polina Rapekta, Pavel Hrouzek, José Cheel and Jaroslav Doubek	205

Continued inside backcover