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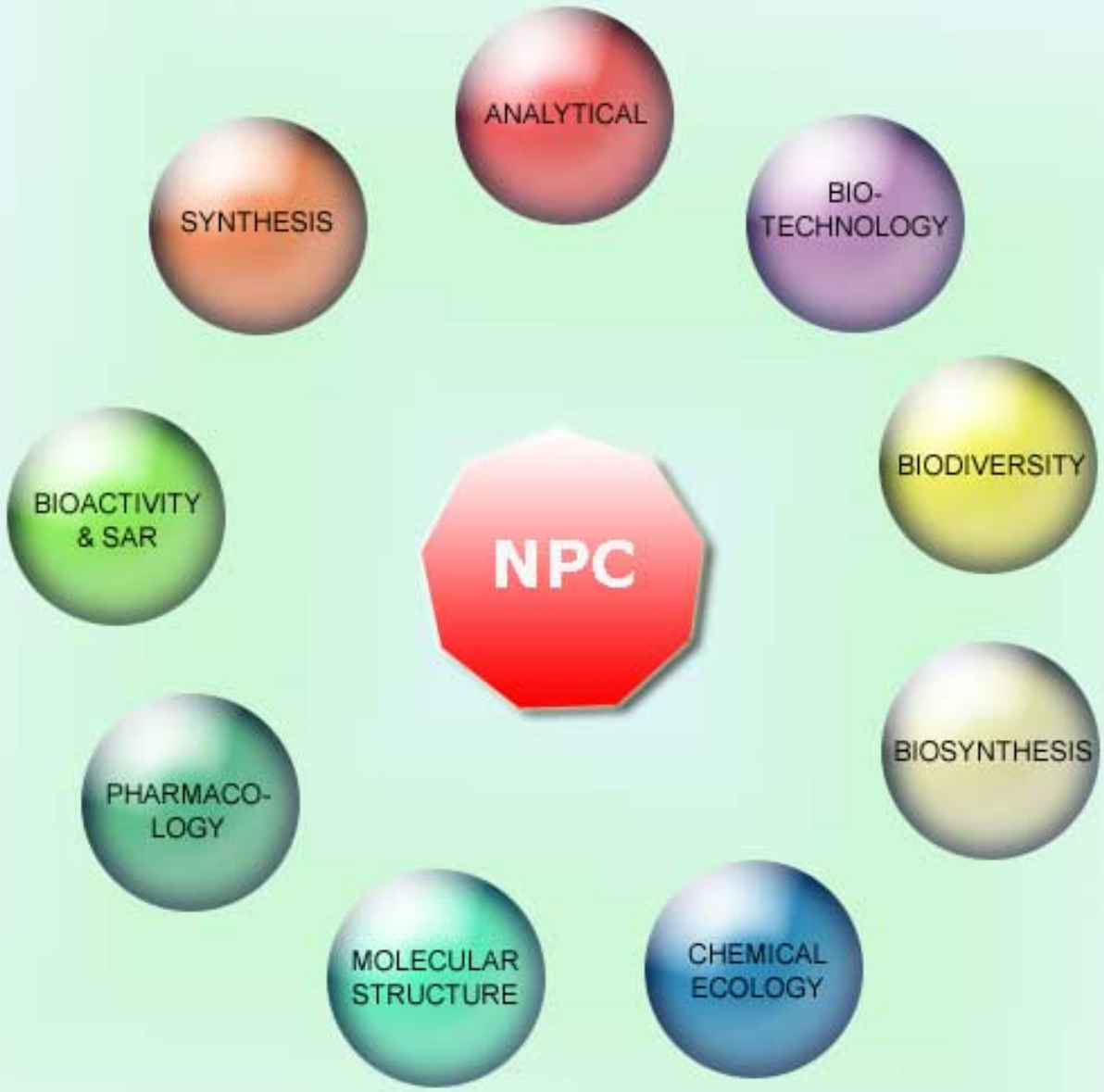

This Issue is Dedicated to
Professor Gerhard Buchbauer
on the Occasion of his 65th Birthday

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Investigation of Anticancer and Antiviral Properties of Selected Aroma Samples

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Anti-tumor and antiviral activities of various aroma samples, including cinnamic acid, β-caryophyllene, caryophyllene oxide, nerolidol (synthetic and natural samples), *trans,trans*-farnesol, farnesol (isomeric mixture), longifolene, geranic acid and a farnesol-rich ylang-fraction were investigated using HeLa and Jurkat cell lines.

Using a CytoTox-96®-Assay we observed strong anti-tumor effects of natural- and synthetic nerolidol-samples at concentrations less than 5 μM. Furthermore, synthetic nerolidol reduced the number of HeLa and Jurkat cells to 50% (CC_{50}) at concentrations which were more than ten times lower compared with the effective doses required to achieve 50% cytotoxicity (ED_{50}). Antiviral studies were performed to investigate inhibitory effects of the compounds on mouse polyomavirus propagation in 3T6 cells. Remarkable inhibition of viral activity was demonstrated by synthetic nerolidol at CC_{50} 3.2 ± 1.5 μM and natural nerolidol at CC_{50} 1.2 ± 0.4 μM All other compounds, however, did not show any inhibition against this virus.

Keywords: antiviral, anticancer, cytotoxicity, nerolidol, farnesol.

Presently, viruses are known to play a key role in the etiology of various tumors [1]. However, severe side effects of established drugs against oncogenic viruses are quite common during therapy, and far less toxic compounds are required [2]. Although anticancer [3] and antiviral properties [4] of aromatic compounds have already been described in the literature, little is known about the possible action of essential oils and their main aromatic components on oncogenic viruses.

Polyomaviruses have been found to be viral agents for oncogenesis and produce a wide range of pathological lesions [5]. The human polyomaviruses (JCV and BK) are ubiquitous and infections often remain asymptomatic, but can cause severe diseases in immunocompromized individuals, especially as a result of AIDS, advanced malignancy or organ transplantation [6]. Modern treatment of diseases caused by human polyomaviruses has quite low effectiveness and often induces complications. Currently, only few scientific data about possible activity of natural compounds against human polyomaviruses are available. Therefore, further research exploring new possible strategies in the treatment of human polyomavirus associated diseases is required.

In the present study, anti-tumor and antiviral potential of various aromatic samples, namely cinnamic acid, β-caryophyllene, caryophyllene oxide, nerolidol (synthetic and natural), *trans,trans*-farnesol,

Table 1: Effect of natural and synthetic aroma samples on viability of HeLa cells.

Compounds	CC_{50} (μM)	ED_{50} (μM)
Caryophyllene oxide	< 30	< 50
β-Caryophyllene	< 15	< 30
Ylang-fraction	< 30	< 50
Nerolidol synthetic	1.5 ± 0.7	< 10
Nerolidol natural	4.2 ± 1.4	< 10
Cinnamic acid	> 100	> 100
Longifolene	< 30	< 50
Geranic acid	> 100	> 100
trans,trans-Farnesol	< 5	< 10
Farnesol (isomer mixture)	< 10	< 10

CC_{50}: sample concentration required to reduce the number of cells to 50%; ED_{50}: effective dose of the sample required to achieve 50% of cytotoxicity for cells

farnesol (mixture of different isomers), longifolene geranic acid and a farnesol-rich ylang-fraction, were investigated on HeLa cells. Jurkat cells were also included in the analyses to account for the possibility of cell-type specific activity of the samples investigated.

As presented in Table 1, effectiveness of these aroma samples on the viability of HeLa cells ranged from high (nerolidol synthetic, CC_{50} 1.5 ± 0.7 μM) to no activity (cinnamic acid and geranic acid, both CC_{50} and ED_{50} > 100 μM). A reduction of HeLa cells, growing as a monolayer, to 50% was observed after applying *trans,trans*-farnesol and nerolidol (natural and synthetic) at concentrations (CC_{50}) less than 5 μM. Moreover, the CC_{50} of synthetic nerolidol in HeLa cells was almost ten times less than the effective dose required to achieve 50% cytotoxicity of the cells (ED_{50}). Farnesol (isomer mixture) and β-caryophyllene demonstrated inhibitory effects on the growth of HeLa cells at concentrations (CC_{50}) less than 15 μM. Since nerolidol synthetic and natural have been demonstrated to have a high activity, additional tests were carried out to determine the precise CC_{50} values of these samples (also repeated for samples of *trans,trans*-farnesol and farnesol mixture). Cinnamic- and geranic acid did not show any activity against either cell line at concentrations less than 100 μM.

To account for the cell type-specific effect of the samples the test was also performed on Jurkat cells. Synthetic nerolidol reduced the number of Jurkat cells, growing in suspension, to 50% at 4.6 ± 2.4 μM (CC_{50}). Nevertheless, the difference between CC_{50} and ED_{50} values for the synthetic nerolidol in Jurkat cells was not statistically significant (data not shown).

In addition, inhibition of mouse polyomavirus production in 3T6 cells was observed for synthetic nerolidol at CC_{50} 3.2 ± 1.5 μM and natural nerolidol at CC_{50} 1.2 ± 0.4 μM. While the effective concentration (CC_{50}) of natural nerolidol against mouse polyomavirus appeared to be more than ten times lower compared with the cytotoxic dose (ED_{50}), the synthetic nerolidol showed a CC_{50} three times lower compared with the ED_{50} (ED_{50} 11 ± 1.8 μM and 10.6 ± 3 μM, respectively). All other aroma samples, except *trans,trans*-farnesol, and farnesol (isomer mixture), which were not tested, did not show any significant antiviral activity in concentrations less than ED_{50} (data not shown).

We have obtained, for the first time, a remarkable reducing effect of natural and synthetic nerolidol and *trans,trans*-farnesol on the viability of tumor cells. Furthermore, antiviral activity of natural and synthetic nerolidol against mouse polyomavirus could be observed. Similarity in the activity against tumor cells between nerolidol and farnesol could be due to their similar chemical structure.

It is obvious that mouse polyomavirus infection is absolutely dependent on host cell transcription and replication machinery [7]. In view of this, our findings about general effects of nerolidol on the viability of tumor cells and mouse polyomavirus infection provide the basis for further investigations about the mechanisms responsible for the activity of nerolidol.

Future research will focus on correlations between different isomer compositions of nerolidol and their activity against tumor cells.

Experimental

Aroma compounds and essential oils: Cinnamic acid, β-caryophyllene, caryophyllene oxide, *trans,trans*-farnesol, and geranic acid were purchased from Sigma-Aldrich (Wien, Austria). Farnesol (isomeric mixture containing *cis,cis*-farnesol 1.3%, *cis,trans* and *trans-cis* farnesol 48.5%, *trans,trans* farnesol 50.2%), farnesol-rich ylang-fraction (containing linalylacetate 0.1%, prenylbenzoate and methylisoeugenol 2.6%, α-farnesene 58.4%, λ-cadinene 4.9%, δ-cadinene 23.1%, cadina-1.4-diene 2.4%, α-cadinene 3.4%), natural nerolidol (> 99% *trans*-farnesol), synthetic nerolidol (contains *cis*-nerolidol 40.7%, *trans*-nerolidol 58.3%, *cis*-dihydronerolidol 0.4% and *trans*-dihydronerolidol

0.6%) and longifolene were purchased from Kurt Kitzing Co. (Wallerstein, Germany). Each aroma compound was dissolved in dimethylsulfoxide (DMSO) and subsequently diluted in the medium. To avoid DMSO toxicity or interference, the maximum concentration of DMSO in the test medium was kept to 0.5%.

Virus and cells: HeLa (human cervix carcinoma) and Jurkat E6.1 (human leukaemic T cell lymphoblast, derived from a patient with acute lymphoblastic leukemia) cell lines were grown in RPMI 1640 medium (SIGMA), supplemented with 2 mM l-glutamine and 10% FBS. Mouse polyomavirus was propagated in 3T6 cells (Swiss albino mouse fibroblasts) using Dulbecco's modified Eagle's medium (DMEM; Sigma) supplemented with 4 mM l-glutamine and 10% FBS. Cells were grown at 37°C in a 5% CO_2-air humidified incubator. For virus infections, mouse polyomavirus (MPyV) strain A2 was used at the multiplicity of infection (MOI) 5 plaque-forming units per cell (PFU/cell).

Virus infection and isolation: 3T6 cells were washed with DMEM and incubated with virus inoculum for 1 h at 37ºC. After that, DMEM supplemented with 10% FBS and containing compounds to be analyzed was added. MPyV was isolated from the cells according to the modified standard protocol [8]. Briefly, cells after repetitive freezing cycles were centrifuged for 40 min at 4300 rpm at 4ºC and the supernatant was collected for further use. The sediment was resuspended in 10 mM Tris HCl (pH 7.4), homogenized (Potter homogenizer) and centrifuged for 40 min at 4300 rpm at 4ºC. The supernatant was mixed with the previous one and centrifuged through 10% sucrose in an ultracentrifuge (Beckman) for 3 h (35,000 RPM, 4ºC). The sediment was resuspended and used for virus titration.

Virus titration: The titer of virus was estimated by immunofluorescence microscopy. Briefly, 3T6 cells grown on coverslips were infected following the procedure as described above. Cells were fixed for 24 h post infection with 4% paraformaldehyde in PBS (15 min), and permeabilized with 0.5% Triton X-100 in PBS (5 min). Immunostaining with primary and secondary antibodies was carried out with monoclonal rat anti-PyV Large T antigen (prepared in our laboratory) and goat anti-rat conjugated with Alexa Fluor-488 dye, respectively.

Cellular toxicity: Cellular toxicity of natural and synthetic aroma compounds was tested using the CytoTox-96® Assay according to the standard manufacturer's protocol. The EC_{50} values were calculated by regression analysis of the dose response curves generated from the data.

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Chemical and Biological Investigations of Essential Oils from Stem Barks of *Enantia chlorantha* Oliv. and *Polyalthia suaveolens* Engler. & Diels. from Cameroon
Maximilienne Nyegue, Paul-Henri Amvam-Zollo, François-Xavier Etoa, Huguette Agnaniet and Chantal Menut **1089**

Evaluation of the Activities of Five Essential Oils against the Stored Maize Weevil
Oluwakemi O. Odeyemi, Patrick Masika and Anthony J. Afolayan **1097**

Physiological and Behavioral Effects of 1,8-Cineol and (±)-Linalool: A Comparison of Inhalation and Massage Aromatherapy
Eva Heuberger, Josef Ilmberger, Engelbert Hartter and Gerhard Buchbauer **1103**

Synergistic and Antagonistic Interactions of Essential Oils on the Biological Activities of the Solvent Extracts from Three *Salvia* species
Guy P. P. Kamatou, Robyn L. van Zyl, Hajierah Davids, Sandy F. van Vuuren and Alvaro M. Viljoen **1111**

Essential Oil Analysis of the Follicles of Four North American *Magnolia* Species
Wolfgang Schühly, Samir A. Ross, Zlatko Mehmedic and Nikolaus H. Fischer **1117**

Essential Oil Composition of *Eryngium campestre* L. Growing in Different Soil Types. A Preliminary Study
Jesús Palá-Paúl, Jaime Usano-Alemany, A. Cristina Soria, M. José Pérez-Alonso and Joseph J. Brophy **1121**

Essential Oil Compounds of *Origanum vulgare* L. (Lamiaceae) from Corsica
Brigitte Lukas, Corinna Schmiderer, Ulrike Mitteregger, Chlodwig Franz and Johannes Novak **1127**

Comparative Study of the Chemical Profiles of the Essential Oils of Ripe and Rotten Fruits of C*itrus aurantifolia* Swingle
Anthony J. Afolayan and Olayinka T. Asekun **1133**

Chemical Variation in the Essential Oil Composition of *Hyptis suaveolens* (L.) Poit. (Lamiaceae)
Paolo Grassi, Marvin José Nuñez, Tomás Sigfrido Urías Reyes and Chlodwig Franz **1137**

Variability of the Volatile Oil Composition in a Population of *Silaum silaus* from Eastern Austria
Remigius Chizzola **1141**

The Bark Essential Oil Composition and Chemotaxonomical Appraisal of *Cedrelopsis grevei* H. Baillon from Madagascar
Miarantsoa Rakotobe, Chantal Menut, Hanitriniaina Sahondra Andrianoelisoa, Voninavoko Rahajanirina, Philippe Collas de Chatelperron, Edmond Roger and Pascal Danthu **1145**

Essential Oil Polymorphism of Hungarian Common Thyme (*Thymus glabrescens* Willd.) Populations
Zsuzsanna Pluhár, Szilvia Sárosi, Ildikó Novák and Gabriella Kutta **1151**

Diversity of Essential Oil Glands of Spanish Sage (*Salvia lavandulifolia* Vahl, Lamiaceae)
Corinna Schmiderer, Paolo Grassi, Johannes Novak and Chlodwig Franz **1155**

Micro-bore Column Fast Gas Chromatography-Mass Spectrometry in Essential Oil Analysis
Peter Quinto Tranchida, Rosaria Costa, Paola Dugo, Giovanni Dugo and Luigi Mondello **1161**

Carbon Isotope Ratio Analysis of Authentic and Commercial Essential Oils of Lemon Balm
Susanne Wagner, Polona Vreca, Albrecht Leis and Herbert Boechzelt **1165**

Accounts/ Reviews

Essential Oil Compounds for Thrips Control – A Review
Elisabeth H. Koschier **1171**

Linalool – A Review of a Biologically Active Compound of Commercial Importance
Guy P. P. Kamatou and Alvaro M. Viljoen **1183**

Limonene - A Review: Biosynthetic, Ecological and Pharmacological Relevance
Paul Erasto and Alvaro M.Viljoen **1193**

Natural Product Communications
2008
Volume 3, Number 7

Contents

Original paper ***Page***

Chemical Composition, Olfactory Evaluation and Antioxidant Effects of the Essential Oil of *Satureja montana* L.
Ivanka Stoilova, Stefanie Bail, Gerhard Buchbauer, Albert Krastanov, Albena Stoyanova, Erich Schmidt and Leopold Jirovetz **1035**

Chemical Composition, Olfactory Evaluation and Antioxidant Effects of an Essential Oil of *Origanum vulgare* L. from Bosnia
Ivanka Stoilova, Stefanie Bail, Gerhard Buchbauer, Albert Krastanov, Albena Stoyanova, Erich Schmidt and Leopold Jirovetz **1043**

Chemical Composition, Olfactory Evaluation and Antioxidant Effects of an Essential Oil of *Thymus vulgaris* L. from Germany
Ivanka Stoilova, Stefanie Bail, Gerhard Buchbauer, Albert Krastanov, Albena Stoyanova, Erich Schmidt and Leopold Jirovetz **1047**

Chemical Composition, Olfactory Evaluation and Antioxidant Effects of the Essential oil of *Origanum majorana* L. from Albania
Erich Schmidt, Stefanie Bail, Gerhard Buchbauer, Ivanka Stoilova, Albert Krastanov, Albena Stoyanova and Leopold Jirovetz **1051**

GC-MS-Analysis, Antimicrobial Activities and Olfactory Evaluation of Essential Davana (*Artemisia pallens* Wall. ex DC) Oil from India
Stefanie Bail, Gerhard Buchbauer, Erich Schmidt, Juergen Wanner, Alexander Slavchev, Albena Stoyanova, Zapriana Denkova, Margit Geissler and Leopold Jirovetz **1057**

Comparative Evaluation of Antimicrobial Activity andComposition of Rose Oils From Various Geographic Origins, in Particular Bulgarian Rose Oil
Velizar Gochev, Katrin Wlcek, Gerhard Buchbauer, Albena Stoyanova, Anna Dobreva, Erich Schmidt and Leopold Jirovetz **1063**

Composition and Antimicrobial Analysis of the Essential Oil of *Litsea laevigata* Nees. (Lauraceae)
Muhammed Arif M, Subbu Raj M, Leopold Jirovetz and Mohamed Shafi P **1069**

Chemical Composition and Antifungal Activity of *Angelica sinensis* Essential Oil against three *Colletotrichum* species
Nurhayat Tabanca, David E. Wedge, Xiaoning Wang, Betul Demirci, Kemal Husnu Can Baser, Ligang Zhou and Stephen J. Cutler **1073**

Development of a Miniaturized 24-well Strawberry Leaf Disk Bioassay for Evaluating Natural Fungicides
Xiaoning Wang, David E. Wedge, Nurhayat Tabanca, Robert D. Johnson, Stephen J. Cutler, Patrick F. Pace, Barbara J. Smith and Ligang Zhou **1079**

Investigation of Anticancer and Antiviral Properties of Selected Aroma Samples
Boris Ryabchenko, Elena Tulupova, Erich Schmidt, Katrin Wlcek, Gerhard Buchbauer and Leopold Jirovetz **1085**

Continued inside back cover