

Aus dem Institut für Hygiene und Umweltmedizin
(Direktor Universitätsprofessor Dr. rer. Nat. Wolfgang Dott)

**Investigation of toxicological effects of various terpenes
on human alveolar adenocarcinoma cell line**

Von der Medizinischen Fakultät
der Rheinisch-Westfälischen Technischen Hochschule Aachen
zur Erlangung des akademischen Grades
eines Doktors der Medizin
genehmigte Dissertation

vorgelegt von
Bülent Izmit

aus
Konak, Türkei

Berichter: Herr Universitätsprofessor
Dr. rer. Nat. Wolfgang Dott

Herr Professor
Dr. med. Gerhard Andreas Wiesmüller

Tag der mündlichen Prüfung: 15.02.2016

D 82 (Diss. RWTH Aachen University, 2016)

Akademische Edition Umweltforschung

Band 51/2016

Bülent Izmit

**Investigation of toxicological effects of various
terpenes on human alveolar adenocarcinoma cell line**

Herausgeber:
Univ.-Prof. Dr. W. Dott
Institut für Hygiene und Umweltmedizin

Shaker Verlag
Aachen 2016

Bibliographic information published by the Deutsche Nationalbibliothek

The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at <http://dnb.d-nb.de>.

Zugl.: D 82 (Diss. RWTH Aachen University, 2016)

Copyright Shaker Verlag 2016

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission of the publishers.

Printed in Germany.

ISBN 978-3-8440-4327-3

ISSN 1437-532X

Shaker Verlag GmbH • P.O. BOX 101818 • D-52018 Aachen

Phone: 0049/2407/9596-0 • Telefax: 0049/2407/9596-9

Internet: www.shaker.de • e-mail: info@shaker.de

Table of Contents

List of Figures.....	III
List of Tables.....	IV
List of Abbreviations.....	V
1. Introduction.....	1
2. Chemical and Biological Background.....	4
2.1 Volatile Organic Compounds.....	4
2.1.1 Terpenes.....	7
2.1.2 α - Pinene.....	9
2.1.3 Eugenol.....	10
2.1.4 d – Limonene.....	12
2.2 Bioavailability.....	14
2.2.1 Respiration.....	14
2.2.2 Biotransformation.....	16
2.3 Biological Response.....	17
2.3.1 Oxidative Stress.....	17
2.3.2 Proinflammatory Stress.....	19
2.4 Legal Regulations.....	22
3. Materials and Methods.....	24
3.1 Human cell line A549.....	24
3.2 Cell count determination.....	30
3.3 Incubation of A549 cells with terpenes and terpenoid like compounds.....	32
3.4 Extraction of intra- and extracellular proteins and enzymes.....	34
3.5 Viability determination of the incubated cells.....	35
3.6 Oxidative Stress.....	37
3.7 Verification of terpene induced proinflammatory Response.....	42
3.8 Analysis for the possible genotoxic effects of terpenes.....	44

4. Results.....	47
4.1 Cell Viability.....	47
4.2 Oxidative Stress.....	50
4.2.1 Superoxide Dismutase.....	50
4.2.2 Catalase.....	57
4.2.3 Total GSH.....	64
4.3 Proinflammatory response.....	71
4.3.1 Interleukin 6.....	71
4.3.2 Interleukin 8.....	78
4.4 Genotoxicity.....	85
5. Discussion.....	86
5.1 Determination of best possible test settings.....	86
5.2 Cytotoxicity.....	89
5.3 Oxidative Stress.....	92
5.4 Inflammatory Stress.....	98
5.5 Genotoxicity.....	101
6. Conclusion.....	102
7. Summary.....	104
List of References.....	105
Picture Credits.....	112
 Appendix.....	 IX
 Acknowledgement.....	 IX
 Erklärung zur Datenaufbewahrung.....	 XI
Eidesstattliche Erklärung gemäß § 5 Abs. (1) und § 11 Abs. (3) 12. der Promotionsordnung.....	XIII
 Curriculum vitae.....	 XV
Conference contributions.....	XVI