

A close-up photograph of several dandelion seed heads in various stages of maturity, with some seeds beginning to disperse. The image is overlaid with a purple-to-pink gradient.

VOLUME I

HANDBOOK OF CHEMICAL AND BIOLOGICAL PLANT ANALYTICAL METHODS

Editor-in-Chief | Kurt Hostettmann

WILEY



Handbook of Chemical and Biological Plant Analytical Methods



Handbook of Chemical and Biological Plant Analytical Methods

VOLUME I

Part One: Sample Preparation and Identification

Part Two: Instrumentation for Chemical Analysis

Editor-in-Chief

Kurt Hostettmann

Honorary Professor at the Universities of Geneva and Lausanne, Switzerland

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VOLUME II

Part Three: Strategies for Selective Classes of Compounds

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VOLUME III

Part Four: Biological Analysis

Part Five: Drugs from Plants

Part Six: Conclusion and Perspectives

Editor-in-Chief

Kurt Hostettmann

Honorary Professor at the Universities of Geneva and Lausanne, Switzerland

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Editorial Board

Editor-in-Chief

Kurt Hostettmann

Honorary Professor at the Universities of Geneva, Lausanne, Nanjing, Shandong and at the
Institute of Materia Medica, Chinese Academy of Science, Shanghai
Invited Professor at Chulabhorn Research Institute, Bangkok, Thailand
Champex-Lac, Switzerland

Editors

Shilin Chen

Institute of Medicinal Plant Development (IMPLAD)
Chinese Academy of Medical Sciences (CAMS)
WHO Collaborating Centre for Traditional Medicine
Beijing
PR China

Andrew Marston

Deceased

Hermann Stuppner

University of Innsbruck
Institute of Pharmacy/Pharmacognosy
CCB - Center for Chemistry and Biomedicine
Innsbruck
Austria

Contents

VOLUME I

List of Contributors xi

Preface xvii

In Memoriam, Andrew Marston xix

Part One

Sample Preparation and Identification 1

1 Selection, Identification, and Collection of Plants 3
Monique S. J. Simmonds

2 Extraction Methodologies: General Introduction 17
Valérie Camel

3 Supercritical Fluid Extraction 43
Seied Mahdi Pourmortazavi, Mehdi Rahimi-Nasrabadi and Somayeh Mirsadeghi

4 New Trends in Extraction of Natural Products: Microwave-Assisted Extraction and Pressurized Liquid Extraction 77
Philippe Christen and Beatrice Kaufmann

5 Solid-Phase Microextraction (SPME) and Its Application to Natural Products 105
M. Abdul Mottaleb, Mohammed J. Meziani and M. Rafiq Islam

Part Two

Instrumentation for Chemical Analysis 129

6 Microscopic Analysis 131
Johannes Saukel and Elisabeth Ginko

7 Thin-Layer Chromatography, with Chemical and Biological Detection Methods 185

Aurélie Urbain and Claudia Avello Simões-Pires

8 HPLC and Ultra HPLC: Basic Concepts 207
Veronika R. Meyer

9 Near-Infrared (NIR) Spectroscopy in Natural Product Research 227
Christian W. Huck

10 Headspace Sampling and Gas Chromatography: A Successful Combination to Study the Composition of a Plant Volatile Fraction 245
Barbara Sgorbini, Cecilia Cagliero, Chiara Cordero, Erica Liberto, Patrizia Rubiolo and Carlo Bicchi

11 Analysis of Natural Products by Capillary Electrophoresis and Related Techniques 277
Markus Ganzera and Anja Krüger

12 LC and LC-MS: Techniques and Applications 307
Nadja Arens, Stefanie Doell and Hans-Peter Mock

13 NMR as Analytical Tool for Crude Plant Extracts 317
Anna R. Bilia

14 NMR of Small Molecules 349
Christoph Seger

15 NMR of Large Molecules	361	23 Identification and Characterization of Trimeric Proanthocyanidins of Two Members of the <i>Rhododendron</i> Genus (<i>Ericaceae</i>) by Liquid Chromatography Multi-Stage Mass Spectrometry	525
<i>Christoph H. Wunderlich, Sarina Grutsch, Martin Tollinger and Christoph Kreutz</i>		<i>Rakesh Jaiswal, Mohamed G. E. Karar and Nikolai Kuhnert</i>	
16 On-Line and At-Line LC-NMR and Related Micro-NMR Methods	379	24 Strategies in the Analysis of Flavonoids	543
<i>Nadine Bohni, Emerson F. Queiroz and Jean-Luc Wolfender</i>		<i>Celestino Santos-Buelga and Ana M. González-Paramás</i>	
17 New Developments of Laser Desorption Ionization Mass Spectrometry in Plant Analysis	411	25 Coumarins – Analytical and Preparative Techniques	569
<i>Andreas Schinkovitz, Denis Séraphin and Pascal Richomme</i>		<i>Krystyna Skalicka-Woźniak and Kazimierz Głowniak</i>	
VOLUME II			
List of Contributors	xi	26 Naphthoquinones and Anthraquinones: Chemical, Analytical, and Biological Overview	595
Preface	xvii	<i>Nahed El-Najjar, Hala Gali-Muhtasib, Pia Vuorela, Arto Urtti and Heikki Vuorela</i>	
In Memoriam, Andrew Marston	xix	27 Xanthoness from Marine-Derived Microorganisms: Isolation, Structure Elucidation, and Biological Activities	611
Part Three Strategies for Selective Classes of Compounds	425	<i>Madalena M. M. Pinto, Raquel A. P. Castanheiro and Anake Kijjoa</i>	
18 Analysis of Plant Oligo- and Polysaccharides	427	28 Sesquiterpenes and Other Terpenoids	633
<i>Wolfgang Blaschek</i>		<i>Eirini Kouloura, Job Tchoumtchoua, Maria Halabalaki and Alexios-Leandros Skaltsounis</i>	
19 Analytical Strategies for Multipurpose Studies of a Plant Volatile Fraction	447	29 Analysis of Plant Saponins	687
<i>Cecilia Cagliero, Barbara Sgorbini, Chiara Cordero, Erica Liberto, Carlo Bicchi and Patrizia Rubiolo</i>		<i>Justyna Krzyzanowska, Mariusz Kowalczyk and Wiesław Oleszek</i>	
20 Strategies for Lipid Analysis	467	30 Cardiotonic Glycosides	709
<i>Irina A. Guschina and John L. Harwood</i>		<i>Liselotte Krenn</i>	
21 HPLC Analysis of Alkaloids	485	31 Plant Steroids: Occurrence, Biological Significance, and Their Analysis	727
<i>Brás Heleno de Oliveira</i>		<i>G. M. Kamal B. Gunaherath and A. A. Leslie Gunatilaka</i>	
22 Identification and Characterization of Hydroxycinnamates of Six <i>Galium</i> Species from the Rubiaceae Family	505	32 Chemical Analysis of Bryophytes	753
<i>Rakesh Jaiswal, Marius F. Matei, Sagar Deshpande and Nikolai Kuhnert</i>		<i>Yoshinori Asakawa, Agnieszka Ludwiczuk and Masao Toyota</i>	

33 Cyclotide Analysis	807	41 Multivariate Data Analysis	915
<i>Susan E. Northfield, Aaron G. Poth, Charlotte D'Souza and David J. Craik</i>		<i>Bieke Dejaegher</i>	
VOLUME III		Part Five	
List of Contributors	xi	Drugs from Plants	947
Preface	xvii	42 <i>In Silico</i>-Guided Strategies for the Discovery and Rationalization of Bioactive Natural Products	949
In Memoriam, Andrew Marston	xix	<i>Petra H. Pfisterer, Daniela Schuster, Judith M. Rollinger and Hermann Stuppner</i>	
Part Four		43 Innovative Strategies in the Search for Bioactive Plant Constituents	967
Biological Analysis	825	<i>Emerson F. Queiroz and Jean-Luc Wolfender</i>	
34 Phenotyping	827	44 High Throughput Screening of Vegetal Natural Substances	987
<i>Qiaosheng Guo and Zaibiao Zhu</i>		<i>Bruno David and Frédéric Ausseil</i>	
35 Identification of Medicinal Plants Using DNA Barcoding	843	45 Mycotoxins Contamination in Food: Alternative Plant Preservatives, Legislation, and Detection Methods	1011
<i>Xiaohui Pang and Shilin Chen</i>		<i>Monica R. Zuzarte, Ana P. Martins, Maria J. Gonçalves and Lúcia R. Salgueiro</i>	
36 Transcriptome Analysis of Medicinal Plants with Next-Generation Sequencing Technologies	847	46 Quality Assessment of Herbal Drugs and Medicinal Plant Products	1039
<i>Shilin Chen and Hongmei Luo</i>		<i>Iqbal Ahmad, Mohd Sajjad Ahmad Khan and Swaranjit Singh Cameotra</i>	
37 Microarray	859	Part Six	
<i>Chang Liu, Haimei Chen, Jianqin Li and Xiaolan Xu</i>		Conclusion and Perspectives	1057
38 Small RNAs	875	47 Conclusion and Perspectives	1059
<i>Shanfa Lu</i>		Index	1061
39 Metabolomics	885		
<i>Jan Schripsema and Denise Dagnino</i>			
40 Proteomics and Its Research Techniques in Plants	903		
<i>Liming Yang, Haibin Xu and Shilin Chen</i>			

Contributors

Iqbal Ahmad *Department of Agricultural Microbiology, Aligarh Muslim University, Aligarh, India*

Nadja Arens *Department of Physiology and Cell Biology, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*

Yoshinori Asakawa *Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, Japan*

Frédéric Ausseil *Institut de Recherche Pierre Fabre, Toulouse, France*

Carlo Bicchi *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italy*

Anna R. Bilia *Department of Chemistry, University of Florence, Sesto Fiorentino (Firenze), Italy*

Wolfgang Blaschek *Department of Pharmaceutical Biology, University of Kiel, Kiel, Germany*

Nadine Bohni *School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland*

Cecilia Cagliero *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italy*

Valérie Camel *AgroParisTech, Paris, France*

Swaranjit Singh Cameotra *Institute of Microbial Technology, Chandigarh, India*

Raquel A. P. Castanheiro *Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), CIIMAR, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal*

Haimei Chen *Institute of Medicinal Plant Development, Chinese Academy of Medical Science, Beijing, PR China*

Shilin Chen *The National Engineering Laboratory for Breeding of Endangered Medicinal Materials, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, PR China*

Philippe Christen *School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland*

Chiara Cordero *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italy*

David J. Craik *The University of Queensland, Brisbane, Queensland, Australia*

Charlotte D'Souza *The University of Queensland, Brisbane, Queensland, Australia*

Denise Dagnino *Grupo Metabolômica, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Brazil*

Bruno David *Institut de Recherche Pierre Fabre, Toulouse, France*

Bieke Dejaegher *Department of Analytical Chemistry and Pharmaceutical Technology (FABI), Vrije Universiteit Brussel (VUB), Brussels, Belgium*

Sagar Deshpande *Jacobs University Bremen, School of Engineering and Science, Chemistry, Bremen, Germany*

Stefanie Doell *Department of Physiology and Cell Biology, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*

Nahed El-Najjar *Department of Physiology and Biophysics, Weill Cornell Medical College, Doha, Qatar*

Hala Gali-Muhtasib *Department of Biology, American University of Beirut, Beirut, Lebanon*

Markus Ganzera *Institute of Pharmacy, Pharmacognosy, University of Innsbruck, Innsbruck, Austria*

Elisabeth Ginko *University of Vienna, Vienna, Austria*

Kazimierz Głowniak *Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Lublin, Poland*

Maria J. Gonçalves *Center for Neuroscience and Cell Biology, Department of Zoology, University of Coimbra, Coimbra, Portugal and Center of Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal*

Ana M. González-Paramás *Facultad de Farmacia, Universidad de Salamanca, Salamanca, Spain*

Sarina Grutsch *University of Innsbruck and Center for Molecular Biosciences Innsbruck (CMBI), Institute of Organic Chemistry, Innsbruck, Austria*

G. M. Kamal B. Gunaherath *The Open University of Sri Lanka, Nugegoda, Sri Lanka*

A. A. Leslie Gunatilaka *The University of Arizona, Tucson, AZ, USA*

Qiaosheng Guo *Institute of Chinese Medicinal Materials, Nanjing Agricultural University, Nanjing, Jiangsu, PR China*

Irina A. Guschina *School of Biosciences, Cardiff University, Cardiff, UK*

Maria Halabalaki *Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Athens, Greece*

John L. Harwood *School of Biosciences, Cardiff University, Cardiff, UK*

Christian W. Huck *Leopold-Franzens University, Innsbruck, Austria*

M. Rafiq Islam *Department of Natural Sciences, Northwest Missouri State University, Maryville, MO, USA*

Rakesh Jaiswal *Jacobs University Bremen, School of Engineering and Science, Chemistry, Bremen, Germany*

Mohamed G. E. Karar *Jacobs University Bremen, School of Engineering and Science, Chemistry, Bremen, Germany*

Beatrice Kaufmann *School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland*

Mohd Sajjad Ahmad Khan *Institute of Microbial Technology, Chandigarh, India*

Anake Kijjoa *ICBAS-Instituto de Ciências Biomédicas de Abel Salazar and CIIMAR, Universidade do Porto, Porto, Portugal*

Eirini Kouloura *Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Athens, Greece*

Mariusz Kowalczyk *Institute of Soil Science and Plant Cultivation, State Research Institute, Pulawy, Poland*

Liselotte Krenn *Department of Pharmacognosy, University of Vienna, Vienna, Austria*

Christoph Kreutz *University of Innsbruck and Center for Molecular Biosciences Innsbruck (CMBI), Institute of Organic Chemistry, Innsbruck, Austria*

Anja Krüger *Institute of Pharmacy, Pharmacognosy, University of Innsbruck, Innsbruck, Austria*

Justyna Krzyzanowska *Institute of Soil Science and Plant Cultivation, State Research Institute, Pulawy, Poland*

Nikolai Kuhnert *Jacobs University Bremen, School of Engineering and Science, Chemistry, Bremen, Germany*

Jianqin Li *Institute of Medicinal Plant Development, Chinese Academy of Medical Science, Beijing, PR China*

Erica Liberto *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italy*

Chang Liu *Institute of Medicinal Plant Development, Chinese Academy of Medical Science, Beijing, PR China*

Shanfa Lu *Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, PR China*

Agnieszka Ludwiczuk *Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Lublin, Poland*

Hongmei Luo *The National Engineering Laboratory for Breeding of Endangered Medicinal Materials, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, PR China*

Ana P. Martins *Center of Pharmaceutical Studies, Faculty of Pharmacy, Health Science Campus, University of Coimbra, Coimbra, Portugal*

Marius F. Matei *Jacobs University Bremen, School of Engineering and Science, Chemistry, Bremen, Germany*

Veronika R. Meyer *Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Protection and Physiology, St. Gallen, Switzerland*

Mohammed J. Meziani *Department of Natural Sciences, Northwest Missouri State University, Maryville, MO, USA*

Somayeh Mirsadeghi *Department of Chemistry, Islamic Azad University, Varamin Pishva Branch, Varamin, Iran*

Hans-Peter Mock *Department of Physiology and Cell Biology, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany*

M. Abdul Mottaleb *Center for Innovation and Entrepreneurship, Northwest Missouri State University, Maryville, MO, USA and Department of Natural Sciences, Northwest Missouri State University, Maryville, MO, USA*

Susan E. Northfield *The University of Queensland, Brisbane, Queensland, Australia*

Wiesław Oleszek *Institute of Soil Science and Plant Cultivation, State Research Institute, Pulawy, Poland*

Brás Heleno de Oliveira *Federal University of Paraná, Curitiba, Brazil*

Xiaohui Pang *The National Engineering Laboratory for Breeding of Endangered Medicinal Materials, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, PR China*

Petra H. Pfisterer *Institute of Pharmacy/ Pharmacognosy and Pharmaceutical Chemistry, and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck, Austria*

Madalena M. M. Pinto *Centro de Química Medicinal da Universidade do Porto (CEQUIMED-UP), CIIMAR, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal*

Aaron G. Poth *The University of Queensland, Brisbane, Queensland, Australia*

Seied Mahdi Pourmortazavi *Faculty of Material and Manufacturing Technologies, Malek Ashtar University of Technology, Tehran, Iran*

Emerson F. Queiroz *School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland*

Mehdi Rahimi-Nasrabadi *Department of Chemistry, Imam Hossein University, Tehran, Iran*

Pascal Richomme *University of Angers, UFR des Sciences Pharmaceutiques, Angers, France*

Judith M. Rollinger *Institute of Pharmacy/ Pharmacognosy and Pharmaceutical Chemistry, and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck, Austria*

Patrizia Rubiolo *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italy*

Lígia R. Salgueiro *Center for Neuroscience and Cell Biology, Department of Zoology, University of Coimbra, Coimbra, Portugal and Center of Pharmaceutical Studies, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal*

Celestino Santos-Buelga *Facultad de Farmacia, Universidad de Salamanca, Salamanca, Spain*

Johannes Saukel *University of Vienna, Vienna, Austria*

Andreas Schinkovitz *University of Angers, UFR des Sciences Pharmaceutiques, Angers, France*

Jan Schripsema *Grupo Metabolômica, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, Brazil*

Daniela Schuster *Institute of Pharmacy/ Pharmacognosy and Pharmaceutical Chemistry, and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck, Austria*

Christoph Seger *University of Innsbruck, Institute of Pharmacy/Pharmacognosy, CCB—Centrum of Chemistry and Biomedicine, Innsbruck, Austria and Institute of Medical and Chemical Laboratory Diagnostics (ZIMCL), University Hospital/Landeskrankenhaus Innsbruck, Innsbruck, Austria*

Denis Séraphin *University of Angers, UFR des Sciences Pharmaceutiques, Angers, France*

Barbara Sgorbini *Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Torino, Italy*

Claudia Avello Simões-Pires *School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland*

Monique S. J. Simmonds *Royal Botanic Gardens, Kew, Richmond, Surrey, UK*

Krystyna Skalicka-Woźniak *Department of Pharmacognosy with Medicinal Plant Unit, Medical University of Lublin, Lublin, Poland*

Alexios-Leandros Skaltsounis *Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Athens, Greece*

Hermann Stuppner *Institute of Pharmacy/ Pharmacognosy and Pharmaceutical Chemistry, and Center for Molecular Biosciences Innsbruck, University of Innsbruck, Innsbruck, Austria*

Job Tchoumtchoua *Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, Athens, Greece*

Martin Tollinger *University of Innsbruck and Center for Molecular Biosciences Innsbruck (CMBI), Institute of Organic Chemistry, Innsbruck, Austria*

Masao Toyota *Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, Japan*

Aur lie Urbain *Pharmacognosy and Bioactive Natural Products, University of Strasbourg, Illkirch, France*

Arto Urtti *Centre for Drug Research, University of Helsinki, Helsinki, Finland*

Heikki Vuorela *Division of Pharmaceutical Biology, University of Helsinki, Helsinki, Finland*

Pia Vuorela *Division of Pharmaceutical Biosciences, University of Helsinki, Helsinki, Finland*

Jean-Luc Wolfender *School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland*

Christoph H. Wunderlich *University of Innsbruck and Center for Molecular Biosciences Innsbruck (CMBI), Institute of Organic Chemistry, Innsbruck, Austria*

Haibin Xu *The Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education, Institute of Medicinal Plant Development, Beijing, PR China*

Xiaolan Xu *Institute of Medicinal Plant Development, Chinese Academy of Medical Science, Beijing, PR China*

Liming Yang *Huaiyin Normal University, Huaian, Jiangsu, PR China*

Zaibiao Zhu *Institute of Chinese Medicinal Materials, Nanjing Agricultural University, Nanjing, Jiangsu, PR China*

Monica R. Zuzarte *Center for Neuroscience and Cell Biology, Department of Zoology, University of Coimbra, Coimbra, Portugal*

Preface

There are many books and book series dealing with chemical and biological methodologies for plant analysis. Thus, was it necessary to publish another book? That was my thought when Martin Röthlisberger from John Wiley & Sons approached me to become the Editor-in-Chief of the present Handbook. I was in the first instance not very enthusiastic and had some hesitations before finally accepting the task. Plants and plant-derived compounds and drugs are becoming more and more popular and also more and more researchers are involved in plant analysis. Quality control of herbal drugs is becoming essential to avoid severe health problems. In addition, in the future, many new drugs will be developed from plant sources. The present Handbook is quite unique as it deals with chemical and biological methodologies for plant analysis. It is a handbook and not an encyclopedia. Thus, it does not present all methods that are available for plant analysis, but a selection of the most important and most accurate ones. Before any analysis, there is an important step involving plant selection and collection, followed by extraction and sample preparation. Several instrumentations for chemical plant analysis are presented with an emphasis on hyphenated techniques such as the coupling between HPLC and mass spectroscopy and HPLC and NMR. A section of this Handbook is devoted to strategies for selective classes of compounds. However, not all classes of plant constituents are reviewed but the most interesting ones such as polysaccharides, saponins, cardiotonic glycosides, alkaloids, terpenoids, lipids, volatile

compounds, and polyphenols (flavonoids, xanthenes, coumarins, naphthoquinones, anthraquinones, proanthocyanidins, etc.). An interesting section deals with biological analysis including phenotyping, DNA barcoding techniques, transcriptome analysis, microarray, metabolomics, and proteomics. The fifth section is devoted to the screening of plant extracts and to strategies for the quick discovery of novel bioactive natural products. Safety assessment of herbal drugs is highly dependent on outstanding chromatographic and spectroscopic methods, which are highlighted here.

The aim of this Handbook is to introduce scientists involved in plant studies and current knowledge of methodologies to various fields of chemically and biochemically related topics in plant research. Emphasis is put on the rapid identification of constituents that could become drugs in the future. When we started work on this Handbook, I had three co-editors to assist me in this task. Unfortunately, one of them passed away, namely Professor Andrew Marston, before the book was completed. In order to honor his memory, this Handbook is dedicated to him, and you will find a short text related to him.

I would like to express my thanks to the two co-editors for their great help in the elaboration of this Handbook and to all the contributors for their collaboration by providing excellent manuscripts.

Dr Kurt Hostettmann
Champex-Lac, Switzerland
July 2014

In Memoriam, Andrew Marston, November 16, 1953 to March 26, 2013

It is a very sad moment for a retired professor to write an obituary for a younger colleague and friend. Andrew studied chemistry at the University College, London, and obtained his BSc degree in 1975. I met him for the first time in the same year when he joined the University of Neuchâtel, Switzerland, as a British Council award holder. He was involved in the research on phytochemistry of gentians and published his first paper with me on flavonoids of *Gentiana pyrenaica*. He not only liked to work in the laboratory but also enjoyed to work in the fields, as he had an excellent knowledge in taxonomy. In fact, we made a beautiful journey together to the French Pyrenees in order to collect the first plant he was working on. This trip was followed later by numerous scientific expeditions all over the world. After Neuchâtel, Andrew went back to England to write his PhD thesis at The University of Liverpool in the field of peptide synthesis, followed by a postdoctoral stay at the German Cancer Research Centre, Heidelberg, Germany, from 1979 to 1983. In October 1983, he joined the Institute of Pharmacognosy and Phytochemistry, University of Lausanne, Switzerland, to work with me on a Swiss National Science Foundation research project for one year. He was a brilliant young scientist, and the initially planned one year stay became a stay of 26 years! Andrew was involved in the isolation of biologically active compounds from plants used in traditional medicine and in the application of new chromatographic techniques for the separation and isolation of plant constituents. He has done pioneering work in the field of

centrifugal partition chromatography, which resulted in the publication of research papers and a couple of review articles. He also achieved original work in the development of enzyme inhibition tests on TLC plates (TLC bioautography), which is useful for the search of acetylcholinesterase inhibitors from plants (Treatment of Alzheimer's disease). For his important contribution in various fields of phytochemistry, Andrew received, in 1994, the prestigious Rhône – Poulenc Rorer Award of the Phytochemical Society of Europe. We published together a book on preparative chromatography techniques, which was translated into Japanese, Chinese, Indonesian, Farsi, and Spanish. He is also co-author of a very complete monography on saponins. In 1994, my institute was transferred from Lausanne to Geneva University where Andrew held the position of *Maître d'enseignement et de recherche* (which corresponds to Senior Lecturer) until my retirement in 2009. When I retired, Andrew decided to look for another job and became Professor of Chemistry at the University of the Free State, Bloemfontein, South Africa. He was conducting in his new job phytochemical investigation on indigenous plants and teaching organic chemistry and natural product chemistry.

Andrew was an outstanding phytochemist, and his work resulted in the publication of more than 150 research papers and 35 review articles and chapters in books. He presented lectures and oral communications in numerous international symposia. He was also teaching in workshops held in Uruguay, Panama, Mexico, Peru, Brazil, Thailand, China, Indonesia,

Zimbabwe, Botswana, and Mali! He passed away on March 26, 2013 in Bloemfontein after a surgery of the brain to control his Parkinson's disease, which resulted in cerebral hemorrhages. He was born in Africa (Northern Rhodesia that became Zambia after independence in 1964) and died in Africa. Moreover, the scientific community has lost a great phytochemist. Everybody will miss Andrew because he was always modest, friendly, and helpful. I shall miss a friend whom I considered as my younger brother.

Dr. K. Hostettmann



Part One

Sample Preparation and Identification

Selection, Identification, and Collection of Plants

Monique S. J. Simmonds

Royal Botanic Gardens, Kew, Richmond, Surrey, UK

1 SELECTION OF PLANTS

An appropriate, well-researched strategy for the selection of plants for a study on natural product or drug discovery is often the key to a successful project. Most studies on natural product provide very little information as to why the specific plants were selected, other than to indicate that the species were selected because they were known to have medicinal or pesticidal properties. In these studies, the authors rarely provide any evidence that the plants being extracted in the laboratory have the medicinal properties attributed to that species in the literature. For example, have the plants been obtained from those that are using them? There are many pitfalls that are common to this field of research, but the proof that these pitfalls have been addressed is not evident from the Material and Methods section of a scientific paper. For example, few papers provide information that enables the researcher to evaluate the robustness of the historical information about the traditional uses of a species, or evidence that the authors read the original papers that describe the traditional uses or collected the plants from those who have traditionally used them. The confusion in the identification of the species was highlighted by Hsu (2006, 2010) who studied the earlier *Materia Medica* in China and reported that Shen Gua (1031–1095) back in 1086 documented the difference between the species as they are prepared in different ways. The literature is

full of examples of assumptions being made about the history and the identification of plants. This short review aims to help highlight the importance of documenting the uses of the plants along with information about the plants being studied and the importance of placing the work on natural product into the context of policies, especially those that support the conservation of the natural resources we study. The majority of examples are from medicinal plant research, but the issues are often common to other natural resources.

Recent work on the development of the antimalarial compound, artemisinin, from *Artemisia annua* L. is now questioning whether the plant used over 2000 years ago in traditional Chinese medicine (TCM) to treat fevers was in fact *A. annua* (cao hao) or another species *Artemisia apiacea* Hance (qing hao)? It would appear that the species that was historically used in TCM to treat conditions now known to be associated with malaria was actually *A. apiacea*. The confusion in the identification of the species was highlighted by Hsu (2006), who studied the earlier *Materia Medica* in China and reported that Shen Gua (1031–1095), back in 1086, documented the difference between the species as they are prepared in different ways. Despite this finding, antimalarial research still focuses on artemisinin isolated from *A. annua* and the cultivation of chemotypes that have the potential to yield high amounts of artemisinin. Although over 600 compounds have been identified in *A. annua*, there is to date very little information

about the chemical potential of *A. apiacea*. The questions can be raised as to why more emphasis has not been placed on the traditionally used species *A. apiacea*, rather than *A. annua*. However, there is no doubt that the isolation of artemisinin from *A. annua* has made a major contribution to the treatment of malaria. Further advances in the use of these species might occur if a comparative study is undertaken of the two species as prepared traditionally. Such a study could include a standard way of extraction the plants (e.g. ethanol extraction) as well as the traditional methods used to make the extractions as well as the traditional formulae (van der Kooy and Sullivan, 2013). The information could assist in highlighting differences in the profile of compounds extracted from the plant that could impact the efficacy of the extracts. The different extracts could be tested through a system biology or pharmacometabonomics approach (Everett, Loo, and Pullen, 2013), in which blood and urine samples of animals or volunteers are analyzed along with the plant material. The information coming from these studies could increase our knowledge about the importance of the complexity of the diversity of compounds in the extracts and how some of these compounds could modulate the enzymes in the different parts of the alimentary channel that influence bioavailability of active compounds (Magalhaes *et al.*, 2012).

A key then to the start of a project is that the researcher should be confident that the plant they propose to study is the correct species, especially if they are going to evaluate the traditional uses of that plant. They should also think about how they are going to check and collate information about the species and how confident are they that the literature they are citing relates to the species they propose to study. This means that they should check with a botanist the identity of the material they propose to work with, as well as the scientific Latin binomial name of the plant. It is also suggested that help is sought to bring together all the relevant names together of the selected species before starting the search. This should include not only the Latin scientific botanical name but also the pharmaceutical names as well as common names. The fact that, currently, in this age of advanced internet searching systems, there is no one central resource that brings all plant names together reflects the complexity of the task (Chan *et al.*, 2012).

The following example illustrates how complex the botanical aspects of a literature review can be. One

of the popular Chinese medicinal plant formulation used in China is Liu Wei Di Huang Wan “Six Flavor Rehmanni,” which contains material from five plants and a fungus. Researchers wanting to collate information about what the activity of the formulation is could undertake a search of the literature using the Pin Yin name of the formulae Liu Wei Di Huang Wan or they could use the names of the plants and fungus used in the formulation. Table 1 illustrates the complexity in undertaking this task. Currently, a medicinal plant could have many scientific names as well as the accepted Latin binomial name and authority given to a species when it was first described. If researchers want to undertake a thorough review of the literature, they will need to have not only all the scientific names but also the common or trivial names of the species. It is also suggested that they include synonyms in the search terms. For example, a quick search of the Web of Science will show that there are more papers about the medicinal uses of the species *Paeonia ostii* T. Hong and J. X. Zhang using the name *Paeonia suffruticosa* Andr. (over 100 papers since the revision of the genus in 1999) than the accepted name *P. ostii* (14 papers since 1999, of which none are about its medicinal uses). It was initially thought that *P. suffruticosa* was a synonym for *P. ostii*, but the recent revision of the genus indicates that *P. suffruticosa* is a different species and not the one cited in the majority of papers (see Table 1 for details).

Collating the names together will enable the researchers to search for information that could relate to the species they propose to study, but how reliable is this information? The identification of plants is a common problem associated with research on the biological activity and chemistry of plants and fungi. The extent of the problem is difficult to establish but it will most likely increase with fewer students being taught traditional taxonomy and plant identification. The development of new mobile applications might assist but the technology is not there yet. The simple question to ask is did someone check the identification of the species being studied? Not all specimens will be easy to identify, especially if when the plant was collected, it was not in flower. One way to help deal with potential problems with the identification of plant is for researchers to keep numbered vouchers of the material they study, these vouchers be kept by their institute, be identified by their number in scientific papers, and be made available to others to check. This is an old tradition used in herbaria around the world and allows others

Table 1 Matrix of plant and fungus species names for guiding literature search and analysis.

Latin scientific name(s) as stated in the Pharmacopoeia of the PRC (English editions) ^{1,2}	Accepted* scientific name ^{3,4,5}	Plant part used in TCM ^{1,2}	Latin pharmaceutical/pharmacopoeia name(s) ^{1,2}	Chinese and Pin Yin names ^{1,2}	Latin scientific synonyms ^{3,4,5}	Official substitute species in Japanese formulations of Liu Wei Di Huang Wan	Unofficial substitutes and adulterants (using Latin scientific names ^(note a) , ordered alphabetically by family)
The following six species are those used in the TCM formula: "Liu Wei Di Huang Wan" ¹							
<i>Alisma orientale</i> (Sam.) Juzep ² (note different and incorrect spelling in the 2005 edition of the Chinese Pharmacopoeia " <i>Alisma orientale</i> (Sam.) Juzep." ⁷)	<i>Alisma orientale</i> (Samuelsson) Juzepczuk ³	Tuber/rhizome	Rhizoma Alismatis ¹ ; Rhizoma ²	泽泻 "Ze Xie" or "Zexie"	<i>Alisma plantago-aquatica</i> Linnaeus var. <i>orientale</i> Samuelsson ³ ; <i>A. plantago-aquatica</i> subsp. <i>orientale</i> (Samuelsson) Samuelsson ⁴	—	None known
<i>Cornus officinalis</i> Sieb. et Zucc. ^{1,2}	<i>Cornus officinalis</i> Siebold and Zuccarini ³	Ripe fruit (processed with yellow rice wine)	Fructus Corni ¹ ; Corni Fructus (processed with wine) ^(note b)	山茱萸 "Shan Zhu Yu" or "Shanzhuyu"	<i>Macrocarpium officinale</i> (Siebold and Zuccarini) Nakai ³ ; <i>Cornus officinalis</i> var. <i>koreana</i> Kitam. ⁴	—	Many species in same or other families reported as local and/or historical unofficial substitutes but international trade uncertain: Berberidaceae: <i>Berberis amurensis</i> , <i>Berberis Poirerii</i> ; Caprifoliaceae: <i>Viburnum schensianum</i> ; Cornaceae: <i>Cornus oblonga</i> ; Rhamnaceae:

(continued overleaf)

Table 1 (Continued)

Latin scientific name(s) as stated in the Pharmacopoeia of the PRC (English editions) ^{1,2}	Accepted* Latin scientific name ^{3,4,5}	Plant part used in TCM ^{1,2}	Latin pharmaceutical/pharmacopoeia name(s) ^{1,2}	Chinese and Pin Yin names ^{1,2}	Latin scientific synonyms ^{3,4,5}	Official substitute species in Japanese formulations of Liu Wei Di Huang Wan	Unofficial substitutes and adulterants (using Latin scientific names ^(note a) , ordered alphabetically by family)
<i>Dioscorea opposita</i> Thunb. ^{1,2}	<i>Dioscorea polystachya</i> Turczaninow ³ (note c)	Tuber/rhizome	Rhizoma Dioscoreae ¹ ; Dioscoreae Rhizoma ²	山药 “Shan Yao” or “Shanyao”	<i>Dioscorea batatas</i> Decaisne ³ ; <i>Dioscorea decaisneana</i> Carrière ³ ; <i>Dioscorea doryphora</i> Hance ³ ; <i>Dioscorea potaninii</i> Prain and Burkill ³ ; <i>Dioscorea rosthornii</i> Diels ¹ ; <i>Dioscorea swinhoei</i> Rolfe ³ ; <i>Dioscorea batatas</i> f. <i>clavata</i> Makino ⁴ ; <i>D. batatas</i> f. <i>daikok</i> Makino ⁴ ; <i>D. batatas</i> f. <i>flabellata</i> Makino ⁴ ; <i>D. batatas</i> f. <i>rakuda</i> Makino ⁴ ; <i>D. batatas</i> f. <i>tsukune</i> Makino ⁴ ;	<i>Official substitute species in Japanese formulations of Liu Wei Di Huang Wan</i>	<i>Unofficial substitutes and adulterants (using Latin scientific names^(note a), ordered alphabetically by family)</i>
							<i>I. Ziziphus mauritiana</i> , <i>Z. jujuba</i> var. <i>spinosa</i> ; Rosaceae: <i>Cerasus pleiocerasus</i> , <i>Malus baccata</i> , <i>Crataegus pinnatifida</i> ; Rubiaceae: <i>Rubia cordifolia</i> Vitaceae: <i>Vitis vinifera</i> , <i>Vitis amurensis</i>