VOLUME I

HANDBOOK OF CHEMICAL AND BIOLOGICAL PLANT ANALYTICAL METHODS

Editor-in-Chief | Kurt Hostettmann

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Handbook of Chemical and Biological Plant Analytical Methods

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VOLUME I

Part One: Sample Preparation and Identification
Part Two: Instrumentation for Chemical Analysis

Editor-in-Chief

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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

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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, xanthones, 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.

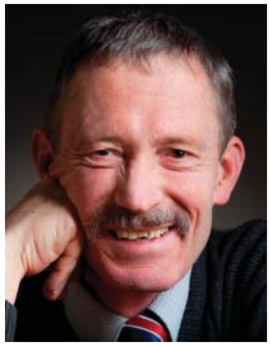
I would like to express my thanks to the two co-editors for their great help in the elaboration of this Handbook and to all the contributors for their collaboration by providing excellent manuscripts.

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

1

Selection, Identification, and Collection of Plants

Monique S. J. Simmonds

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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 (quing 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

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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. siffruticosa* 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* 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)
The following six species are those used in the TCM formula: "Liu Wei Di Huang Wan"[1]							
Alisma orientale (Sam.) Juzep[2] (note different and incorrect spelling in the 2005 edition of the Chinese Pharmacopoeia "*Alisma orientalis* (Sam.) Juzep.")	*Alisma orientale* (Samuelsson) Juzepczuk[3]	Tuber/rhizome	Rhizoma Alismatis[1]; Alismatis Rhizoma[2]	泽泻 "Ze Xie" or "Zexie"	*Alisma plantago-aquatica* Linnaeus var. *orientale* Samuelsson[3]; *A. plantago-aquatica* subsp. *orientale* (Samuelsson) Samuelsson[4]	—	None known
Cornus officinalis Sieb. et Zucc.[1,2]	*Cornus officinalis* 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"	*Macrocarpium officinale* (Siebold and Zuccarini) Nakai[3]; *Cornus officinalis* var. *koreana* Kitam.[4]	—	Many species in same or other families reported as local and/or historical unofficial substitutes but international trade uncertain: Berberidaceae: *Berberis amurensis*., *Berberis Poiretii*; Caprifoliaceae: *Viburnum schensianum*; Cornaceae: *Cornus oblonga*; 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)
							1. Ziziphus mauritiana, *Z. jujuba* var. *spinosa*; Rosaceae: *Cerasus pleiocerasus*, *Malus baccata*, *Crataegus pinnatifida*; Rubiaceae: *Rubia cordifolia* *Vitaceae*: *Vitis vinifera*, *Vitis amurensis*
Dioscorea opposita Thunb.[1,2]	*Dioscorea polystachya* Turczaninow[3;(note c)]	Tuber/rhizome	Rhizoma Dioscoreae[1]; Dioscoreae Rhizoma[2]	山药 "Shan Yao" or "Shanyao"	*Dioscorea batatas* Decaisne[3]; *Dioscorea decaisneana* Carrière[3]; *Dioscorea doryphora* Hance[3]; *Dioscorea potaninii* Prain and Burkill[3]; *Dioscorea rosthornii* Diels[1]; *Dioscorea swinhoei* Rolfe[3]; *Dioscorea batatas* f. *clavata* Makino[4]; *D. batatas* f. *daikok* Makino[4]; *D. batatas* f. *flabellata* Makino[4]; *D. batatas* f. *rakuda* Makino[4]; *D. batatas* f. *tsukune* Makino[4];		