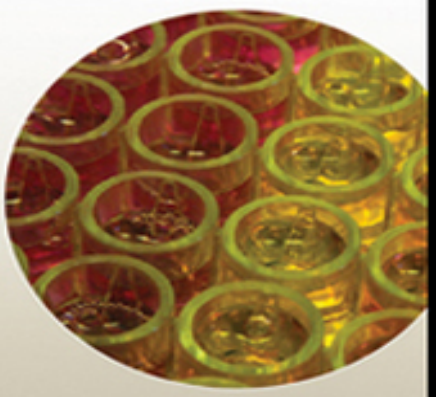
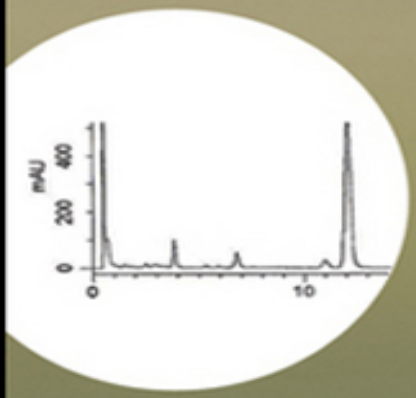
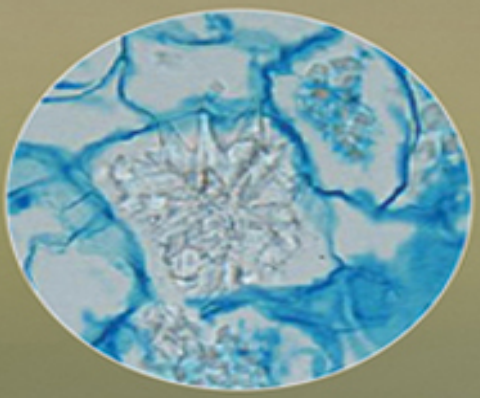
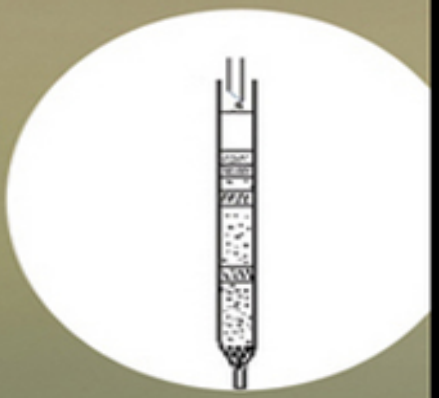


TRADITIONAL HERBAL MEDICINE RESEARCH METHODS

Identification, Analysis, Bioassay, and Pharmaceutical and Clinical Studies







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Willow J.H. Liu

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Dedication

This book is dedicated to all who are interested in traditional herbal medicines and willing to make their contribution to people's health with their efforts.

I first want to thank all of the professors in China, Germany, and the United States who have trained me or with whom I have worked; in chronological order, they are Professor Song-Song Yang, Xin-Sheng Yao, Rudolf Bauer, Koji Nakanishi, Norman R. Farnsworth, John M. Pezzuto, Judy L. Bolton, and Gail B. Mahady. Special acknowledgment is given to all of the authors of this book for contributing their time and knowledge, as well as to Dr. Hong-Jie Zhang for introducing some of them to me. Acknowledgment is also given to all of my previous colleagues and friends who have provided me with generous academic support.

And most of all, I want to thank my husband, Zhuo Chen, for his understanding and all unsung support to my career, and my daughter, Emily (Chen Chen), for her time on editing this book. They have been traveling with me to different countries and cities throughout the world, accompanying me in both body and soul. This book is also a special present to my lovely little son Derek, a precious gift sent from God while I was preparing this book.

Preface

After giving a presentation on the topic of “Modern Research on Traditional Herbal Medicine” in the American Chemical Society national meeting (September 2006, San Francisco), I received an e-mail from John Wiley & Sons asking if I would write a book with the same title.

Research on traditional herbal medicine involves botany, chemistry, biology, pharmacology, toxicology, clinical trials, and other disciplines. Chemical composition and biological or biochemical activities of many herbs have been studied by researchers in universities and pharmaceutical companies for purposes of investigation or new drug development. So far, there have been many books introducing functions or actions of herbs. Books on the chemistry of herbal medicines (often called phytochemistry), biochemistry, biology, and pharmacology of herbal medicines are also available. But there is no book giving a full description of all aspects of herbal research and development.

The purpose of research on traditional herbal medicine is not only for new drug development, but also for quality control and mechanism study of herbs. Unlike screening for new drug candidates simply using one or two bioassay tests, exploration of the mechanisms of traditional herbal medicines is much more complex. It requires close cooperation between scientists from many disciplines to unveil the secrets of the herbal kingdom. For maximal cooperation, pharmacologists, biologists, chemists, and clinical doctors need to have basic knowledge of the cooperating fields. To scientists who are very knowledgeable in modern science and have extensive research experience, their knowledge about the applications and theories of traditional medicines they are studying, for example, traditional Chinese medicine (TCM) and Ayurveda, might be limited. For this reason, their research designs for these

herbs may simply copy those for new drug development. As a consequence, the results may not be accurate due to either inappropriate design of extract methods, insufficient experiment duration in animal study, or lower concentration of samples used for bioassay tests. This is why studies using the same assay for the same herb from different labs have often reported different results.

This book introduces the methodology of collection and identification of herbal materials, extraction and isolation of compounds from herbs, *in vitro* bioassay, *in vivo* animal test, toxicology, and clinical trial for herbal research. It is not written as a literature review. Instead, it introduces the basic content and methodology of each research field and the keys for the study of herbal medicine. The purpose of this book is to help scientists who are interested in the study of traditional herbal medicine gain a broader view of herbal medicine and knowledge about its research. I hope this book can be a bridge to provide scientists in different fields with basic information and knowledge about the progress of herbal study and to help them avoid unnecessary mistakes during their studies.

As for background information on my relationship with traditional herbal medicine, I received my B.S. in Chinese Herbal Medicine, M.S. in pharmacognosy, and Ph.D. in natural product chemistry in succession from Shenyang, China. My doctoral supervisor was Xinsheng Yao of Shenyang Pharmaceutical University, a well-known phytochemist and academician in China. I left China as a professor of phytochemistry at Liaoning University of Traditional Chinese Medicine and traveled to Germany as an AvH Research fellow. There I worked in the lab of Rudolf Bauer at Duesseldorf University, a world-renowned expert in plant medicine, in particular of Echinacea, and an aficionado of Chinese herbs. I focused on bioassay screening and standardization of herbal medicines. Later I moved to New

York and worked in the lab of Koji Nakanishi at Columbia University, and then to Chicago, where I worked with Norman Farnsworth and John Pezzuto at the UIC/NIH Center for Botanical Dietary Supplement Research in Women's Health at the College of Pharmacy, University of Illinois at Chicago. The project there was the mechanism study of herbs for treating women's menopause symptoms, using *in vitro* bioassays and *in vivo* animal tests.

Unfortunately, I became afflicted with rheumatoid arthritis while I was in Chicago. My wrist gave me so much pain that I was too weak to even open a reagent bottle. Even while being treated with Western medicine, I was once paralyzed and could not get out of bed. For health reasons, I thus had to leave Chicago's harsh weather for California, and started to treat myself with Chinese herbs and acupuncture, in addition to treatment with Western medicine, while working in a research lab for pharmaceutical analysis. In my spare time, I taught Chinese herbology, TCM nutrition, as well as modern pharmacology and nutrition, at various schools of acupuncture. A few years later, I opened my own clinic of herbs and acupuncture. Since then, I have been treating patients with my combined knowledge on the functions of traditional herbs and their modern biological and pharmacological activities, meanwhile developing herbal products based on the efficacy of herbal formulas in clinical application. Research, teaching, plus clinical practice strongly consolidated my knowledge on both traditional and modern medicine, and helped me review TCM theories more deeply and from multiple perspectives.

My research on herbs in the past 25 years has told me that they work in a way that differs from modern drugs: the effect is not from one single compound in an herb, but is a synergetic result from many components working on many targets. And researchers should not be disappointed if their results show that the most bioactive compounds screened

from an herbal extract in a bioassay are popular second metabolites in plants. Examples include flavonoids, fatty acids, or amines.

The successful treatment of a variety of diseases in my clinic with Chinese herbal formulas has reminded me of what I had first learned during college: the effective treatment with Chinese herbs is mostly based on formulas composed of several or more individual herbs, rather than single ones, and the formulation of a Chinese herbal prescription is guided by theories of TCM. But most scientists conducting research on traditional herbal medicine today are either unaware of or are ignoring this.

My research in China, Germany, and the United States has extended my knowledge of phytochemistry, analytical chemistry, biochemistry, biology, and pharmacology and experience with extraction, isolation, identification, and analysis of compounds in herbs and their bioassay screening and mechanism study with *in vitro* and *in vivo* tests. This is the reason that I boldly accepted the invitation to write this book. To make each chapter in this book more authoritative, I invited several experts from different fields in China to write some chapters. In the process of editing, necessary rewriting, rearrangement, additions, and clarification of contents were made with the agreement from authors of each chapter. However, due to limited space, it is impossible to cover all aspects or give detailed information in each chapter. I hope this book will work as a guideline for new scientists working with modern technologies and help them to explore more secrets in the treasury of traditional medicines.

Willow J.H. Liu

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Abbreviations

AAS
atomic absorption spectrometry
AEs
adverse effects
AFLP
amplified fragment length polymorphism
AFS
atomic fluorescence spectrometry
AIC
Akaike Information Criterion
ALP
alkaline phosphatase
AMD
age-related macular degenerative disease
ANOVA
analysis of variance
AP
alkaline phosphatase
APCI
atmospheric pressure chemical ionization
API
atmospheric pressure ionization
AP-PCR
arbitrarily primed polymerase chain reaction
APPI
atmospheric pressure photoionization
ASTM
American Society for Testing and Materials

BMD
bone mineral density
CAG
coronary angiography
CAM
complementary and alternative medicine
CCCD
China Certification Committee for Drugs
CD
circular dichroism
CE
capillary electrophoresis
CHD
coronary heart disease
CHL
Chinese hamster lung cell
CI
chemical ionization
CID
collision-induced dissociation
CIOMS
Council for International Organizations of Medical Sciences
CNPIC
China National Pharmaceutical Industry Corporation Limited
CNS
central nervous system
COSY
chemical shift correlation spectroscopy
COX
cyclooxygenase
CP

cyclophosphamide

CPC

centrifugal partition chromatography

CQS

comprehensive quality systems

DAD

diode array detector

DCC

droplet countercurrent

DEPT

distortionless enhancement by polarization transfer

DMEM

Dulbecco’s modified Eagle’s medium

DMSO

dimethyl sulfoxide

DOPAC

3,4-dihydroxyphenylacetic acid

DPD

deoxypyridinoline

DPPH

2,2-diphenyl-1-picrylhydrazyl

E

enzyme

E_2

estradiol

ECD

electrochemical detector

ECG

electrocardiogram

ECL

enhanced chemiluminescence

EFPIA
European Federation of Pharmaceutical Industries Associations
EI
electron ionization
EIA
enzyme immunoassay
EIS
enzyme-inhibitor-substrate complex
ELISA
enzyme-linked immunosorbent assay
ELS
evaporative light scattering
ELSD
evaporative light scattering detector
EMEA
European Medicines Agency
ER
estrogenic receptor
ERB
Ethical Review Board
ERE
estrogen-responsive element
ERT
estrogen replacement therapy
ES
enzyme-substrate complex
ESI
electrospray ionization
EU
European Union

FAB
fast atom bombardment
FBS
fetal bovine serum
FC
flash chromatography
FCPC
fast centrifugal partition chromatography
FD
field desorption
FDA
Food and Drug Administration
FDCA
Federal Food, Drug, and Cosmetic Act
FI
field ionization
FLARE
fragment length associated repair enzyme
FOB
functional observatory battery
FT
Fourier transform
FT-ICR
Fourier transform ion cyclotron resonance
FTMS
Fourier transform mass spectrometry
GAP
good agriculture practice
GABA
γ-aminobutyric acid
GC

gas chromatography
GCP
good clinical practice
GE
gel electrophoresis
GEP
good extracting practice
GLP
good laboratory practice
GMP
good manufacturing practice
GOT
glutamate oxaloacetate transaminase
GPT
glutamate pyruvate transaminase
GSLS
Ginseng stem and leaf saponins
GTP
guanosine triphosphate
HBV
anti-hepatitis B virus
HHS
Department of Health and Human Services
5-HIAA
5-hydroxyindoleacetic acid
HILIC
hydrophilic interaction liquid chromatography
HMBC
heteronuclear multiple bond correlation
HMQC
heteronuclear multiple quantum coherence

HPLC
high-performance liquid chromatography
HRT
hormone replacement therapies
HSCC
high-speed countercurrent
HSQC
heteronuclear single quantum coherence
HT
serotonin
HTS
high-throughput screening
HVA
homovanillic acid
IBS
irritable bowel syndrome
ICH
International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP-MS
inductively coupled plasma mass spectroscopy
IEC
independent ethics committee
IND
investigational new drug
IR
infrared
IRB
institutional review board
ISSR

inter-simple sequence repeats
ITS
internally transcribed spacer
JPMA
Japan Pharmaceutical Manufacturers Association
LC
liquid chromatography
LD
lethal dose
LDL
low-density lipoprotein
LH
luteinizing hormone
LhRh
luteinizing hormone releasing hormone
LIT
linear ion trap
LOD
limit of detection
LOQ
limit of quantitation
LPH
lipotropic hormone
LPLC
low-pressure liquid chromatography
LS
light scattering
LSD
least significant difference
MAE
microwave-assisted extraction

MALDI
matrix-assisted laser desorption
MBC
metastatic breast cancer
MEM
minimum essential medium
MHLW
Ministry of Health, Labor, and Welfare
MOH
Ministry of Health
MPLC
medium-pressure liquid chromatography
MRM
multiple-reaction monitoring
MS
mass spectrum; mass spectrometer; mass spectrometry
MTD
maximum tolerated dose
MTS
3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4- sulfophenyl)-2H-tetrazolium
MTT
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW
molecular weight
NAD^+
nicotinamide adenine dinucleotide
NADH
reduced form of nicotinamide adenine dinucleotide
$NADP^+$
nicotinamide adenine dinucleotide phosphate

NADPH
reduced form of nicotinamide adenine dinucleotide phosphate
NCCAM
National Center for Complementary and Alternative Medicine
NCI
National Cancer Institute
NDA
new drug application
NEI
neuroendocrine-immune network
NIH
National Institutes of Health
NIR
near-infrared
NIRS
near infrared spectrometer; near infrared spectrometry
NLM
National Library of Medicine
NMR
nuclear magnetic resonance
NOAEL
no-observed-adverse-effect level
NOE
nuclear Overhauser effect
NOESY
nuclear Overhauser effect spectroscopy
NP–LC
normal phase liquid chromatography
ODS

octadecasilica
OHRP
Office for Human Research Protections
ORAC
oxygen radical absorbance capacity
ORD
optical rotatory dispersion
ORR
objective response rate
OVX
ovariectomized rat model
PB
particle beam
PBS
phosphate buffer saline
PC
paper chromatography
PCR
polymerase chain reaction
PD
pharmacodynamics
PE
phosphatidylethanolamine
PK
pharmacokinetics
PLE
pressurized liquid extraction
PMS
phenazine methosulfate
pQCT
peripheral quantitative computed tomography

PQR
product quality review
PR
progestin receptor
PRMA
Pharmaceutical Research and Manufacturers of America
PTLC
preparative thin layer chromatography
QA
quality assurance
QC
quality control
QOL
quality-of-life
QRM
quality risk management
QT
Q wave and T wave in ECG
QU
quality unit
RACE
rapid amplification of cDNA ends
RAPD
random amplified polymorphic DNA
RDA
retro-Diels–Alder
RFLP
restriction fragment length polymorphism
RI
refractive index
RP–LC