Shailendra S. Gurav · Nilambari S. Gurav
Editors

Indian Herbal Drug Microscopy

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Shailendra S. Gurav
Department of Pharmacognosy
Government College of Pharmacy
Karad, Maharashtra, India

Nilambari S. Gurav
Department of Pharmacognosy
Sudhakarrao Naik Institute of Pharmacy
Pusad, Maharashtra, India

ISBN 978-1-4614-9514-7 ISBN 978-1-4614-9515-4 (eBook)
DOI 10.1007/978-1-4614-9515-4
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013954404

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Dedicated to our beloved Aai, Anna, and Anu

Foreword

The use of plants as a source of medicine is as old as humanity. Medicinal plants have always played a significant role in treating illness or preventing disease. Over the past several decades, scientific literature and popular media articles on adverse drug effects increased the interest in natural products by the general public. Major issues in plant drug research are proper identification, authentication, and evaluation of the plants. Pharmacognostical and phytochemical evaluation plays a major role in this context.

Anatomical study of plants plays a vital role for their identification and authentication. The knowledge of microscopic details of plants in crude and powder form is vital for the evaluation of medicinal plants in every way. This book on "Indian Herbal Drug Microscopy" will be very helpful in this context for the identification and evaluation of medicinal plants. The book contains several chapters ranging from stepwise procedure for sectioning of plant material to histochemical staining techniques and anatomy of 40 well-known and medicinally important plants like Arjuna, Ashoka, Ashwagandha, Cinchona, Cinnamon, Ginger, Kurchi, Rauwolfia, Turmeric, Tulsi, and Vasaka with hand-drawn colored microscopic images of crude drugs and their magnified powder microscopic characters.

I appreciate the efforts put forward by the authors to develop this laboratory manual, which is an outcome of their experience and hard work. I hope this not only will be very helpful for students but also will be an essential reference for anyone involved in the fields of phytomedicine, traditional herbal remedies, pharmaceutical sciences, and natural product research.

Kolkata, India — Pulok K. Mukherjee, Ph.D., F.R.S.C.

Preface

The past decade has witnessed the introduction and implementation of current Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates, and finished products of botanical origin to overcome adulteration and misidentification of herbal drugs. The initial step in quality control of medicinal plants is confirming the authenticity of the desired species via a variety of techniques such as macro- and microscopic identification and chemical analysis. The botanical control by microscopic examinations, using histological identification, is still most preferred as a rapid and inexpensive technique.

Today there are few laboratory manuals and practical handbooks which highlight microscopy of crude drugs only. However, our book differentiates from others in the context of hand-drawn colored microscopic images of crude drugs and their powder characters as observed under a microscope after magnification. There may be many omissions and commissions, so the author sincerely wishes the kind cooperation of every reader in correcting them.

This work is the output of a number of years of experience in training imparted to pharmacy students in the identification of herbal drugs. It describes today's knowledge of some important botanical microscopic characters of the whole, fragmented, and powdered herbal drugs studied. While referring to this book to authenticate a given sample, it will be necessary to make microscopic preparations of the plant material under study in order to compare the structures present with those drawn and described herewith. All the drawings in this book have been made by us after observation of microscopic preparations by standard techniques from previously authenticated samples, in our laboratory. In preparing the drawings our purpose is to illustrate all the diagnostic as well as microscopic characters which play a crucial role in authentication of plant species. The color diagrams are sufficiently clear, exemplifying that anyone can easily match the characters seen under the microscope with the drawn diagrams. Practical aspects of sectioning and histochemical staining techniques are also described as separate chapters. It also makes the book user friendly for analysts working in pharmaceutical concerns manufacturing herbal products.

I hope our present attempt will be helpful for students, teachers, and researchers of pharmacy and *Ayurveda* as well as analysts in the herbal and Ayurvedic industry.

Karad, Maharashtra, India — Shailendra Gurav
Pusad, Maharashtra, India — Nilambari Gurav

Acknowledgements

It is always hard to list comprehensively all those whose influence has been felt and whose assistance has been liberally sought in the gestation of this manuscript. This book is an output of a number of years of experience in training imparted to pharmacy students in the identification of herbal drugs.

The editors are particularly grateful to Dr. Arun Patil, Dr. Shrikant Tilloo, and Dr. Kishorkumar Burade for their association with the work at various stages and for the helpful comments during the process. The editors warmly appreciate the arduous and dedicated artwork put forth by Mr. Atul Ahire without whom this book was never possible. The editors are also thankful to Dr. Asha Arora, Head of Biotechnology Department, B.N.P.G. College, Udaipur, Rajasthan, India; Mrs. Shubhada Nikharge, Founder Trustee of "Save Rani Bagh Botanical Garden Foundation," Mumbai, India; and Dr. Sandip Hate for their help by providing actual photos of plants for this manuscript. We are very much thankful to Dr. Pulok Mukherjee, Director, School of Natural Product Studies, Jadavpur University, Kolkata, for his appreciation and recommendation in the form of "Foreword" for our book. The editors take this opportunity to thank Dr. Dadasaheb Kokare, Dr. Prashant Puranik, Dr. Vijay Gulkari, and Dr. Santosh Bhujbal for their support at every stage in the preparation of this manuscript. The editors like to express their gratitude to all their colleagues working in different departments for their timely help and moral support.

Finally, the editors extend their appreciation to their parents Aai, Anna, and Baba without whose blessings it was never possible to bring this dream into reality and their little princess "Anu" to whom this book is dedicated.

Karad, Maharashtra, India — Shailendra Gurav
Pusad, Maharashtra, India — Nilambari Gurav

Content

Contributors

Kishor Burade Government College of Pharmacy, Karad, Maharashtra, India

Nilambari S. Gurav Sudhakarrao Naik Institute of Pharmacy, Pusad, Maharashtra, India

Shailendra S. Gurav Government College of Pharmacy, Karad, Maharashtra, India

Arun Patil Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj University, Nagpur, Maharashtra, India

Shrikant Tilloo Gurunanak College of Pharmacy, Nagpur, Maharashtra, India

Chapter 1
Introduction

Shailendra Gurav and Nilambari Gurav

The use of herbal medicines throughout the world has become more and more popular in recent years. Among these, India and China are the most upcoming and blooming countries right now. Extensive use of herbal medicine poses two new problems, with international implications, which increase the importance of fast, accurate, and efficient means of authenticating herbs. First, the growing market for herbal medicines worldwide has engendered many international trading companies and generated an increase in counterfeit herbs and herbs of questionable quality. Second, herbal medicines are often taken as combinations (most of the time) which generate unique problems of authentication, such as determining if there is species confusion of different herbs sharing one name or one herb using different names and if the correct herbal medicine has been included in a particular proprietary medicine.

Adulteration and misidentification of herbal drug can cause serious health problems to consumers, as well as publicity and legal headaches for the pharmaceutical industry. Many poisoning incidents caused by misuse or confusion of herbal medicines have raised international concern for authentication of herbal medicines to their safe and effective use. The past decade has witnessed the introduction and institution of current Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates, and finished products of botanical origin.

The first step in quality control of medicinal plants is ensuring the authenticity of the herbal medicines, and today, there are a variety of methods available to authenticate herbal medicines, ranging from simple morphological examination to physical and chemical analysis and DNA molecular biology. However, each method

S. Gurav (✉)
Government College of Pharmacy, Karad, Maharashtra, India
e-mail: shailugurav@rediffmail.com

N. Gurav
Sudhakarrao Naik Institute of Pharmacy, Pusad, Maharashtra, India
e-mail: nilagurav@rediffmail.com

S.S. Gurav and N.S. Gurav (eds.), *Indian Herbal Drug Microscopy*,
DOI 10.1007/978-1-4614-9515-4_1,

has drawbacks and advantages. In difficult or critical cases, sometimes two or several methods are applied for the authentication of herbal medicines.

Microscopy permits the identification of herbal drugs and the detection of individual components of a mixture by examining their unique features like histological structures, cells, and cell contents. It is of great value in case of comparative analysis of broken and powdered herbal products because in such cases most of the morphological diagnostic features are lost. The powdered crude drugs can be identified based on the form, the presence, or the absence of different cell types based on their cytomorphological characters, e.g., parenchyma, collenchyma, fibres, stone cells, vessels, trichomes, secretory cells, and epidermal cells.

For several years, the magnifying glass and the microscope were the only possible methods for the analytical evaluation of herbal drugs. Advances in microscope technology have improved the accuracy and capabilities of microscopy as a tool of botanical identification.

Different types of microscopic techniques which can be used for the pharmacognostic studies include light microscopy (LM), polarizing microscopy, phase-contrast microscopy, and scanning electron microscopy (SEM). However, ordinary light microscopy is still the most common method for primary authentication. It has been commonly used in the authentication of herbal medicines in India and many other countries because of its virtues of requirement of small amount of sample, fast speed, and economy. Furthermore, herbal medicines are mostly low-cost medicine, which should not be raised in price just because of the application of unnecessary highly sophisticated methods for authentication. Indian Ayurvedic Pharmacopoeia (First Edition 1964) and Ayurvedic Formulary of India (First Edition 1966) clearly show that in India microscopic techniques have been used in the authentication of herbal medicines for a long time. Outside India, the pharmacopoeias of other countries also record the microscopic characteristics of their herbal medicines, for example, European Pharmacopoeia, British Pharmacopoeia, United States Pharmacopeia, Japanese Pharmacopoeia, Chinese Pharmacopoeia, and Vietnamese Pharmacopoeia.

Though there are few laboratory manuals and practical handbooks in market which highlight microscopy characters of crude drugs, present book differentiates from others in the form of hand-drawn colour microscopic images of crude drug and its powdered characters as they observed under microscope after magnification. There may be many omissions and commissions, so the author sincerely wishes the kind cooperation of every reader in correcting them.

In the present book, the drugs have been selected taking into consideration the pharmacognosy course of different institutions of pharmacy in India. The drugs have been initially authenticated from the botany department and then microscopy of same was performed in our laboratory by standard techniques. Powdered drugs before use were sieved through a no. 60 mesh and further used for microscopy study.

It describes today's knowledge of some important botanical microscopic characters of the whole, fragmented, and powdered herbal drugs studied. When using the present book to authenticate a given sample, it will be necessary to make microscopic

preparations of the material in order to compare the structures present with those drawn and described herewith. All the drawings in the present book have been made by us after observation of prepared microscopic preparations by standard techniques from previously authenticated samples, in our laboratory.

Chapter 2
Sectioning Methods

Shailendra Gurav, Nilambari Gurav, and Arun Patil

1 Introduction

Plant anatomy is a basic core subject in the study of biology, especially plant biology. In the study of plant structure, it is important to recognise that there is a fundamental difference between plant and animal development. Thorough knowledge of the structure of plant cells and tissues is the prerequisite for a realistic interpretation of morphology, physiology, and phylogeny. Moreover, it is also essential to solve many important everyday problems such as the identification of unknowns and food contaminants. Therefore in order to learn about plant structures, it is important to take hands on some of the simple techniques that are useful in the study of plant structures.

2 Necessary Material

The variety of instruments can be used depending upon the nature of work. The microscope is the most indispensable instrument used in laboratory which helps to increase the resolving power of human eye which fails to recognise objects lying closer between 0.01 and 0.25 mm. Dissecting and compound microscopes are most

S. Gurav (✉)
Government College of Pharmacy, Karad, Maharashtra, India
e-mail: shailugurav@rediffmail.com

N. Gurav
Sudhakarrao Naik Institute of Pharmacy, Pusad, Maharashtra, India
e-mail: nilagurav@rediffmail.com

A. Patil
Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj University, Nagpur, Maharashtra, India
e-mail: profarunpatil@gmail.com

S.S. Gurav and N.S. Gurav (eds.), *Indian Herbal Drug Microscopy*,
DOI 10.1007/978-1-4614-9515-4_2,

commonly used, whereas binocular, phase-contrast, electron, and fluorescent are other types of microscopes used as per requirement. However, it is more convenient to prepare a small kit containing good and sharp razor blade, a fine hairbrush, watch glasses, Petri dishes, clean slides, coverslips, two fine long handle dissecting needles, a pair of forceps, glass droppers, a pair of sharpened pencils, pencil eraser, a clean and soft handkerchief, etc. Other supplies such as filter papers, lens paper, and lens cleaner for slides and microscope lens should be available in the laboratory.

A set of different staining solutions in dropper bottles, pipettes with rubber bulbs, a large water bottle, and a tray of necessary materials once arranged can be used throughout the technique. The reagents and stains can be replenished as and when required.

3 Freehand Sectioning Methods

Most plant parts are too thick to be mounted intact and viewed with a microscope. In order to study the plant anatomy, sections have to be made so that enough light can be transmitted through the specimen to resolve cell structures under the microscope. A freehand section is the most common and simple method of preparing specimens for microscopic viewing. This method allows one to examine the specimen in a few minutes. It is also suitable for a variety of plant materials, such as soft herbaceous stems and small woody twigs.

Different sections can be obtained from a stem, root, or stolon, depending on the plane of cutting, each section revealing the details from a different angle. Transverse section is obtained by cutting along the radial plane of a cylindrical portion of the stem or root or stolon and perpendicular to the long axis. Tangential longitudinal section is a section cut along the long axis parallel to a tangent, whereas radial longitudinal section is a section cut along the long axis and the cutting plane passing through the long axis and radius.

In general it is found that transverse sections are easier than longitudinal sections as the internal structures are clearly identified in the same. The good transverse sections can be obtained by cutting plant material, to be sectioned at right angle to the long axis of the cells. The fixation of materials is generally not required for temporary preparations. More and more practice, patience, and perhaps one's inherent skill are the prerequisite for this technique.

3.1 Steps

1. Sit comfortably with your forearms resting on the bench and your elbows close to your sides.
2. Have a new sharp double-edged razor blade. To minimise the risk of cutting oneself, cover one edge of the razor blade with masking tape.

3. Hold the plant material firmly against the side of the first finger of any hand (left or right) by means of the thumb. Keep the first finger as straight as possible, while the thumb should kept well below the surface of the material out of the way of the razor edge.
4. Put the drop of water on the razor so as to reduce the friction during cutting as sections can float onto the surface of the blade. Take the razor blade in any hand (left or right) and place it on the first finger of the left hand (or right hand), more or less at a right angle to the specimen.
5. Now move the razor quickly across the top of the material in such manner to give the material a drawing cut in a single complete stroke. This minimises the friction while passing the razor blade through the specimen. Use more and more uniform strokes to get several sections at a time. Sections will certainly vary in thickness. However, there will be usable ones among the thick sections.

   (Note: During sectioning, a number of sections should be cut at the same time without worrying about section thickness at this time. Thick sections are also usable unless they are not obliquely sectioned. One can cut a number of sections without moving the material or the thumb just by slightly and progressively increasing the pressure with the razor blade on the first finger and simultaneously exerting increasing pressure onto the specimen by the thumb. It is advised to start cutting with the razor blade right at the surface of the specimen rather than against the side of the material. In case of the root and stem (as they have a radial symmetry), it is not necessary that a section should be complete, as long as it includes a portion of the tissues from the centre to the outer edge of the specimen.)

6. Transfer sections to water in watch glass using a brush (not a forceps or needle).
7. Select and transfer the thinnest sections (the more transparent ones) onto a clean glass slide, and put two to three drops of chloral hydrate solution on it, and heat the slide very gently by passing to and fro over a low flame. When bubbles start to appear, stop heating, and add a drop of glycerine–water solution to avoid drying of the preparation and crystallisation of chloral hydrate.
8. To apply the coverslip, hold it at an angle and touch the glycerine-water drop with one edge. Lower the coverslip slowly to avoid air bubbles.

   (Note: In case of thin leaves and tiny roots, i.e., delicate and hard to hold specimens, additional support can be used to facilitate hand sectioning. In such cases tissue pieces can be inserted into a small piece of pith such as a potato tuber or carrot or radish root. Once it is sure, the tissue is firmly in place, and the hand sectioning technique can be applied. Without supporting material longitudinal sections are also difficult to obtain as small stem and root pieces are not easy to hold firmly with one's finger. However, by cutting a v-shaped notch into the pith support, sectioning can be done.)