

# **Analytical Methods for Medicinal Plants and Economic Botany**

**2nd Edition**

**M. Daniel  
Denni Mammen**



Published by:

**SCIENTIFIC PUBLISHERS (INDIA)**

5A, New Pali Road, P.O. Box 91

Jodhpur 342 001, India

E-mail: [info@scientificpub.com](mailto:info@scientificpub.com)

Website: [www.scientificpub.com](http://www.scientificpub.com)

**Print:** Second Edition, 2023

All rights reserved. No part of this publication or the information contained herein may be reproduced, adapted, abridged, translated, stored in a retrieval system, computer system, photographic or other systems or transmitted in any form or by any means, electronic, mechanical, optical, digital, by photocopying, recording or otherwise, without written prior permission from the publisher. Any breach will attract legal action and prosecution without further notice.

**Disclaimer:** While every effort has been made to avoid errors and omissions, this publication is being sold and marketed on the understanding and presumption that neither the editors (or authors) nor the publishers nor the printers would be liable in any manner whatsoever, to any person either for an error or for an omission in this publication, or for any action to be taken on the basis of this work. Any inadvertent discrepancy noted may be brought to the attention of the publisher, for rectifying it in future editions, if published.

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the editors and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The editors and publisher have attempted to trace and acknowledge the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission and acknowledgement to publish in this form have not been obtained. If any copyright material has not been acknowledged please write and let us know so that we may rectify it.

**Trademark Notice:** Publications or corporate names may be trademarks, and are used only for identification and explanation in bonafide intent without intent to infringe.

© First Edition 2016, Daniel, M. & M. Denni

ISBN: 978-93-94645-36-3

eISBN: 978-93-94645-38-7

Printed in India

---

## **PREFACE (Second Edition)**

---

It is heartening to see that this book is widely accepted by Students and Teachers of Phytochemistry, Biochemistry, Botany and Molecular biology and thus was sold out within a span of four years. The feedback received was that the book contains simple experiments which can be performed easily even in a poorly equipped laboratory.

During the last four years, two years and more were influenced negatively by fear of Corona and during the lockdown times, almost all college and University research laboratories remained shut. Only Microbiological and Biotechnological laboratories were functioning, that too driven up by a desire to find out a vaccine for Covid virus. Therefore, there was no much development in the field of plant analytical methods and thus this book remains up to date even now.

We are overawed when teachers ring us up and ask us questions they have and which students ask them when they perform the experiments based on this book. That shows a genuine desire to learn these methods for their benefit. Many academic institutions like Universities and colleges have drafted courses based on this book. The phytochemical methods are much needed in studying medicinal plants in Ayurveda, Pharmacy courses, Botany, Biochemistry and Biotechnology and many research projects are being planned at Undergraduate, Post-Graduate and Research levels.

The emphasis on Alternative Medicine drives people to look to plants as safe ways to combat pathogens and to increase immunity. The role of neglected compounds like simple Phenolics, flavonoids and tannins in influencing various biochemical parameters in body is much understood now and this also contribute to screening plants for such compounds.

**M. Daniel &  
Denni Mammen**



---

## PREFACE (First Edition)

---

Plant chemistry has become an integral part of every discipline in plant sciences, alternative medicines (Ayurveda and Siddha), biotechnology, pharmacognosy and organic chemistry. Study of plant chemistry at the molecular level has revolutionized the existing concepts on the structure and function of plants and its products. The rapid strides achieved in extraction methods, chromatography and spectrophotometry in the past few decades have made the study of molecular components of plants feasible which occur even at miniscule. However botanist and scientist dealing with quality control processes for medicinal plants lack the availability of the data.

The international herbal market is worth 61 billion dollars wherein China's share is 4.9%. In spite of having a rich heritage of Ayuervedic literature and a wide range of medicinal plant species, India's contribution to international herbal market is negligible (0.61%). Our products are not acceptable to be used in the western market because of poor quality of our preparations. This is because 80% of the raw material used in Ayurvedic or Herbal industries are adulterated. Adulteration is committed due to unavailability of a desired plant (may be due to overexploitation) in a locality or collection of the wrong plant by unskilled collectors or confusion on the identity due to various vernacular names used in different parts of the country. The absence of a reliable quality control of raw materials with the manufacturers is an important drawback which intentionally prompts many suppliers to provide, either unsuitable plant materials or their extracts. In addition, non-scientific extraction methods followed by many industries that produce formulations, which are poor in the composition of their active compounds. All the above issues result in low acceptability and reliability in Indian as well as the global scenario. The need of the hour is to equip the industry personnel with right and reliable methods of assessment for assessing the genuineness of either the plant or the extract supplied. The present book is a step in this direction.

Study of Economic Botany, which deals with the utilization of plants and plant materials by humans, can be improved by introducing phytochemistry and analytical methods in its curriculum. Economic relevance of plants is because of the bioactive compounds present in them. Study of plants with regard to their composition elevates Economic Botany to the rank of a scientific study, instead of the 'story telling' method practiced so far. The economic products, say either spices or resins, can be studied in university and college laboratories and many elementary methods of analysis can be included as laboratory exercises. Such an approach gives a better exposure to the usefulness of plants and its products. In such a context we propose this book '*A Phytochemical Approach to Economic Botany*'. In this book, the chemistry of plants useful to humans is dealt with in detail with a brief introduction on the chemistry of plant products. A separate laboratory handbook, '*Methods in Plant Chemistry and Economic Botany*' containing several laboratory experiments on

qualitative and quantitative analysis of selected plants and their products was published last year. The former book is revised, enlarged and published as '*The Useful Herbs of The Planet Earth*' [Scientific Publishers (India), Jodhpur, 2013] and this edition is the thoroughly revised form of the latter.

In this book, all available information on the nature and distribution of various natural products in the plant kingdom is provided. Therefore, this book can serve as a text book on phytochemistry as well. A brief chapter on general methods of extraction, principles and instruments used in various chromatographic methods such as Paper Chromatography, Thin Layer Chromatography, Gas Chromatography and High Pressure Liquid Chromatography and UV/Visible spectrophotometry is provided—at the beginning. Simple procedures, which could be executed in a less equipped rural college laboratory as well as GC and HPLC procedures of a large number of plant products, which include various phytochemicals are included. A number of methods have been tested several times in author's laboratory. A person with an elementary knowledge of laboratory techniques in chemistry should be able to carry out these experiments. We are hopeful that such a measure will enable the improvement of quality regulation in research standard on plants. These experiments can be incorporated into the curricula of both undergraduate and postgraduate courses of Pharmacy, Ayurveda, Biotechnology, Biochemistry or Economic botany. For a phytochemist (and to a chemotaxonomist) this book will provide details as to how to acquire standards of plant chemicals. A comparison can be done by a researcher to judge the nature of unknown compound with the given known compounds in the book.

We are thankful to Kiran Poonia and Skariah John in helping us type the parts of this manuscript. We express our sincere gratitude to The Scientific Publishers, Jodhpur, India, for doing an excellent job of printing and processing this treatise.

Comments, suggestions and criticism aimed at improving the scope of this book are welcome.

21 May, 2016

**M. Daniel &  
Denni Mammen**

---

## How to get the best from this book

---

Almost the entire spectrum of phytochemicals are presented in this book and experiments dealing with the chemistry, isolation, identification and quantification of each group are included. A novice student of chemistry may find it tough to start with, but a student having at least a subsidiary level background at B.Sc. level can pick up the procedures without much difficulty. Most of the experiments presented here are easy to perform especially those dealing with PC or TLC as well as titrations, but when one gets into the GC and HPLC analyses, the original papers are to be referred to get the entire gamut of events unfolding clear and easy to follow.

The **standard compounds** needed for co-chromatography or for standard graphs can be procured from the authentic suppliers. Many a times they may not be easy on pocket. In such cases a student can search out the original paper isolating the compound for the first time, follow it ditto with the same material and same processes and lo, your standard compounds are ready for you. A number of large scale isolation procedures which were followed in past are included which may be followed when a large amount of standard compound is desired.

For a researcher **initiating work on a new plant** for its chemical constituents, a literature survey on related plants of the same family or other species of the same genus will give good enough indications on the compounds to be expected. If any related plant is already studied, note down the procedures followed in that plant and follow the same procedures for the plant at hand. If your new plant is synthesizing the same compound your identification is easy and authentic. But if the compounds are different but falls within the same group (say alkaloids, because they give positive test for alkaloids), the chromatographic behavior can give you a rough idea on the nature of difference.

It is always advisable to **cross check the procedures**, the reagents prepared and sequence of events followed. If you do not get a correct result in one attempt, redo it after checking the entire procedure critically; lest you might have overstepped one apparently insignificant procedure.

A word of caution! Please see that the **plant material taken up for the study is the genuine one**. If needed, seek the help a good taxonomist who can identify plants correctly.

Perfection comes with experience. Therefore the more times you do an experiment, the more correct your procedures are. Wish you all an excellent academic and research carrier.





---

# CONTENTS

---

*Preface*

*iii- iv*

*How to get the best from this book*

*v*

<b>1. Methods of Extraction and Characterization</b>	<b>1 - 37</b>
<b>1.1 Extraction</b>	<b>1- 6</b>
<b>1.1.1. Cold Extraction</b>	<b>1</b>
a. Typical cold extraction	1
b. Percolation	2
c. Maceration	2
d. Ultrasonic extraction	2
<b>1.1.2. Hot Extraction</b>	<b>2- 4</b>
a. Infusion	3
b. Refluxing	3
c. Soxhlet's extraction	3
d. Microwave assisted extraction	3
<b>1.1.3 Special methods of Extraction</b>	<b>4-6</b>
a. Supercritical fluid extraction	4
b. Accelerated solvent extraction	5
<b>1.2. Sample purification and concentration</b>	<b>6-8</b>
1. Liquid-Liquid Extraction	6
2. Solid phase extraction (SPE)	6
3. Matrix solid phase dispersion	7
4. Concentration	7
5. Crystallization	8
<b>1.3. Solvents used for extraction</b>	<b>8-10</b>
1. Petroleum ether	9
2. Chloroform	9
3. Methanol/Ethanol	9
4. Water	9
5. DMSO	10
5. Tetrahydrofuran	10

<b>1.4. Isolation and Characterization</b>	<b>10-35</b>
1.4.1. <b>Paper Chromatography</b>	<b>11-17</b>
a. 2-D Paper Chromatography	16
b. Preparative Paper Chromatography	17
1.4.2. <b>Thin Layer Chromatography</b>	<b>17-21</b>
a. Preparative TLC	21
1.4.3. <b>Gas Chromatography</b>	<b>21-27</b>
1. Derivatization of non-volatile compounds	22
2. GC columns	22
a. damage to columns	24
b. Column contamination	24
3. Split/Splitless and On-Column Gas Chromatographic Injectors	25
4. Retention Time.	26
1.4.4. <b>High Performance Liquid Chromatography</b>	<b>27-32</b>
1. HPLC columns	27
2. Stationary phases	27
a. Absorption Mode	28
b. Reversed Phase Mode	28
c. Normal Bonded Phase Mode	29
d. Chiral Bonded Phases	29
e. Silica gel Endcapping	29
3. Guard Columns	29
4. Pre Columns	30
5. Mobile phases	30
6. Column cleaning and regeneration	30
7. Detectors	30
a. Detector cells	31
b. UV and DAD detectors	31
c. Refractometric detectors	31
d. Light scattering devices	31
e. Electrochemical detectors	32
1.4.5. <b>Hyphenated and Specialised Systems</b>	<b>32</b>
1.4.6. <b>Ultraviolet and Visible spectrophotometry</b>	<b>32-37</b>
<b>2. Carbohydrates</b>	<b>38-71</b>
<b>2.1. Monosaccharides</b>	<b>38-54</b>
2.1.1. <b>Properties of monosaccharides</b>	<b>42</b>
2.1.2. <b>Sugar derivatives</b>	<b>42</b>
a. Sugar alcohols	42

b. Sugar acids	44
c. Sugar amines	45
<b>2.2. Disaccharides</b>	45
<b>2.3. Analytical methods</b>	47
<b>2.3.1. Chromatographic analysis of sugars and sugar derivatives</b>	47
a. Paper Chromatographic analysis of sugars	47
b. TLC of sugars	48
c. Gas Chromatography of sugars and related compounds	49
d. HPLC of sugars	50
e. PC analysis of floral nectar of Angiosperms	50
f. Preparation of sugar osazones	51
g. PC of Sugar alcohols	51
h. PC of sugar acids	52
i. Estimation of sugars by Iodimetry	53
<b>2.4. Polysaccharides</b>	<b>55-71</b>
<b>2.4.1. Starch and Inulin</b>	55
a. Isolation of starch from potato	57
b. A general method for the isolation of starch	57
c. Acid hydrolysis of starch	58
d. Isolation of inulin from <i>Dahlia</i> tubers	58
<b>2.4.2. Pectin and Dietary fibre</b>	59
a. Pectin from orange peel	60
b. Isolation of dietary fibre of foods	61
<b>2.2.4. Gums and Mucilages</b>	62
a. Galactomannans from guar seeds	63
b. Agar from Red algae	64
c. Alginic acid from Brown algae	64
<b>2.2.5. Structural Polysaccharides : Cellulose</b>	65
a. Estimation of cellulose in Wood.	67
b. Cellulose triacetate	68
c. Cellulose trinitrate	69
d. Regenerated Cellulose	70
da. Regeneration with phosphoric acid	70
db. Regeneration with Schweitzer's reagent	70
e. Amyloid and Parchment paper	70
f. Purification of cotton cellulose	70
<b>3. Amino acids and Proteins</b>	<b>72-85</b>
<b>3.1. Amino acids: protein and non-protein amino acids</b>	<b>72-81</b>

3.1.1. <b>Protein amino acids</b>	72
a. Properties of amino acids	73
aa. Reactions due to carboxyl group	73
ab. Reactions due to amino group	74
3.1.2. <b>Non-protein amino acids</b>	75
3.1.3. <b>Peptides</b>	78
3.1.4. <b>Analytical methods</b>	79
a. Extraction of amino acids	79
b. PC of amino acids	79
c. TLC of amino acids	80
d. GC of amino acids	80
e. HPLC of amino acids	81
f. Automatic amino acid analysers	81
g. Analysis of peptides	81
<b>3.2 Proteins</b>	81-85
a. Plant storage proteins	82
b. Tests for proteins	83
c. Estimation of proteins in plant materials by Folin-Phenol method	84
d. Isolation of proteins from ground nut seeds	85
e. Paper chromatographic separation of arachin and conarachin	85
<b>4. Fats and Oils</b>	<b>86-95</b>
a. Extraction of oils	88
b. Saponification value of oils	88
c. Acid value of oils	90
d. Iodine value of oils	90
e. Phospholipids TLC	92
f. GC of fatty acids	92
g. HPLC of lipid classes	93
h. HPLC of fatty acids	93
i. Isolation of trimyristicin and myristicin from nutmeg	93
j. Hydrolysis of triacyl lipids	94
<b>5. Terpenoids</b>	<b>96-140</b>
<b>5.1 Mono and sesquiterpenoids, Volatile oils</b>	<b>97-108</b>
5.1. 1. <b>Monoterpenoids</b>	97
5.1. 2. <b>Sesquiterpenoids</b>	98
5.1. 3. <b>Volatile oils</b>	99
a. Extraction of volatile oils	100

b. TLC of mono- and sesquiterpenes	101
c. TLC of monoterpene glycosides	101
d. TLC of volatile oils	101
e. TLC of sesquiterpene lactones	102
f. TLC methods for selected sesquiterpenes:	102
fa. Parthenolide from Feverfew	102
fb. Pyrethrin from Pyrethrum	104
fc. Artemisinin from <i>Artemisia annua</i>	104
g. GC of volatile oils	105
h. HPLC of mono- and sesquiterpenoids	105
<b>5.2. Iridoids</b>	106
a. Extraction of iridoids	107
b. PC of iridoids	107
c. TLC of iridoids	107
d. HPLC of iridoids	108
<b>5.3. Diterpenoids</b>	<b>108-113</b>
a. Extraction and purification of diterpene fractions	109
b. Detection of diterpenoids	109
c. TLC and HPLC of selected diterpenes	109
ca. Tanshinones from Danshen	109
cb. Ginkgolides from <i>Ginkgo biloba</i>	110
cc. Stevioside from <i>Stevia rebaudiana</i>	111
cd. Taxol from <i>Taxus brevifolia</i>	112
ce. Andrographolide from <i>Andrographis paniculata</i>	113
<b>5.4. Triterpenoids</b>	<b>114-132</b>
<b>5.4.1. Phytosterols</b>	116
a. Column Chromatography of phytosterols	117
b. TLC of phytosterols	117
c. GC of phytosterols	117
d. HPLC of phytosterols	117
e. Boswellic acid from Frankincense	117
ea. TLC of boswellic acids	118
eb. HPLC of boswellic acids	118
<b>5.4.2. Ecdysteroids</b>	118
a. TLC of ecdysteroids	118
b. HPLC of ecdysteroids	118
<b>5.4.3. Brassinosteroids</b>	119
a. HPLC of brassinosteroids	119

5.4.4. Cucurbitacins	120
a. HPLC of cucurbitacins	120
5.4.5. Limonoids and Quassinoids	120
a. TLC of limonoids	121
b. HPLC of limonoids	122
5.4.6. Withanolides	122
a. TLC of withanolides	122
b. HPLC of withanolides	123
5.4.7. Saponins	123
a. Extraction of saponins	124
b. TLC of Saponins	124
c. HPLC of some selected saponins:	125
ca. Hydrocotylosides <i>Centella asiatica</i>	125
cb. Ginsenosides from Ginseng	125
d. Extraction and isolation of saponins of <i>Smilax</i> and <i>Asparagus</i>	126
e. Saponins of Liquorice	126
f. Saponins from soapnut	127
g. Isolation of randialic acid, scopoletin and mannitol from <i>Randia</i> bark	128
h. A general procedure for isolation of sapogenins	128
5.4.8. Cardiac glycosides	129
a. Cardenolides	130
aa. TLC of cardenolides	130
ab. HPLC of cardenolides	130
ac. Cardiac glycosides of <i>Digitalis</i> , TLC	131
b. Bufadienolides	132
ba. TLC of bufadienolides	132
bb. HPLC of bufadienolides	132
<b>5.5. Carotenoids</b>	<b>132-135</b>
a. Extraction of carotenoids	133
b. TLC of carotenoids	134
c. PC of carotenoids	134
d. HPLC of carotenoids	134
e. Lycopene from tomato	134
f. Capsanthin from chillies	136
<b>5.6. Polyisoprenoids - Rubber and Gutta</b>	<b>136</b>
1. Isolation of rubber and gutta from latex	137
2. Petrocrops and Petroleum substitutes	137

3. Extraction of Biocrude	139
<b>6. Alkaloids</b>	<b>141-192</b>
<b>6.1. General methods of extraction and testing of alkaloids</b>	<b>143</b>
a. Extraction of alkaloids	143
b. Testing the plant samples for alkaloids	144
c. General reagents for alkaloids	144
<b>6.2. Alkaloidal amines</b>	<b>145-147</b>
<b>6.2.1. Alkaloids of <i>Ephedra</i></b>	<b>145</b>
a. Extraction of alkaloids	146
b. Tests for ephedrine	146
c. TLC of <i>Ephedra</i> Alkaloids	146
d. HPLC of Ephedrine	146
e. Estimation of ephedrine: Titrimetry	146
<b>6.2.2. Isolation of aegeline and aegelinine from <i>Aegle</i></b>	<b>147</b>
<b>6.3. Indole alkaloids</b>	<b>147-161</b>
<b>6.3.1. <i>Vinca</i> Alkaloids</b>	<b>152</b>
a. Extraction of alkloids	153
b. TLC of <i>Vinca</i> alkloids	153
c. Column chromatography <i>Vinca</i> alkloids	153
d. HPLC of <i>Vinca</i> alkloids	153
<b>6.3.2. Alkaloids of <i>Rauwolfia</i></b>	<b>154</b>
a. Extraction of alkaloids	154
b. TLC of <i>Rauwolfia</i> alkloids	154
c. HPLC of <i>Rauwolfia</i> alkloids	155
<b>6.3.3. Alkaloids of <i>Strychnos</i></b>	<b>155</b>
a. Isolation of strychnine and brucine	155
b. Tests for <i>Strychnos</i> alkaloids	156
c. TLC of <i>Strychnos</i> alkaloids	156
d. HPLC of <i>Strychnos</i> alkloids	156
e. Determination of strychnine and brucine separately by titration	156
<b>6.3.4. Ergot alkaloids</b>	<b>157</b>
a. TLC of ergot alkaloids	157
b. HPLC of ergot alkloids	158
c. Isolation of ergotoxine and ergotinine	158
d. Tests for ergot alkaloids	159
e. Estimation of total ergot alkaloids	159
<b>6.3.5 Alkaloids of <i>Peganum</i></b>	<b>160</b>

a. Isolation of alkaloids	160
b. TLC of <i>Peganum</i> alkloids	160
c. HPLC of <i>Peganum</i> alkloids	160
d. Estimation of total alkaloids	161
<b>6.4. Isoquinoline alkaloids</b>	<b>161-168</b>
6.4.1. <b>Alkaloids of <i>Tinospora</i></b>	163
a. Isolation of alkloids	164
b. TLC of <i>Tinospora</i> alkloids	164
c. HPLC of <i>Tinospora</i> alkloids	164
6.4.2. <b>Alkaloids of Barberry</b>	164
a. Extraction	164
b. TLC of Barberry alkloids	165
c. HPLC of Barberry alkloids	165
6.4.3. <b>Alkaloids of <i>Papaver somniferum</i> Linn.</b>	165
a. TLC of <i>Papaver</i> alkloids	166
b. HPLC of <i>Papaver</i> alkloids	166
6.4.4. <b>Alkaloids of <i>Alangium</i></b>	166
a. PC of <i>Alangium</i> alkaloids	166
b. TLC of <i>Alangium</i> alkaloids	166
c. HPLC of <i>Alangium</i> alkaloids	167
d. Estimation of total alkaloids	167
e. Isolation of alkaloids	168
<b>6.5. Purine alkaloids</b>	168
a. Caffeine from tea	168
b. Tests for caffeine	169
c. TLC of alkaloids of coffee, tea and cocoa	169
d. HPLC of caffeine	169
e. Large scale isolation of caffeine	169
<b>6.6. Pyridine-piperidine alkaloids</b>	170
6.6.1. <b>Alkaloids of <i>Areca</i> nut</b>	170
a. TLC of <i>Areca</i> alkloids	170
b. HPLC of <i>Areca</i> alkaloids	170
c. Isolation of arecoline	170
d. Estimation of arecoline – Titrimetry	171
6.6.2. <b>Tobacco Alkaloids</b>	171
a. TLC of tobacco alkaloids	172
b. Gas chromatography of tobacco alkaloids	172
c. HPLC of tobacco alkaloids	173



d. Isolation of nicotine and nor-nicotine	173
e. Tests for nicotine	173
f. Estimation of total alkaloids – Titrimetry	173
<b>6.6.3. Alkaloids of <i>Piper</i></b>	174
a. PC of pepper alkloids	174
b. TLC of pepper alkloids	174
c. HPLC of pepper alkaloids	175
<b>6.6.4. Alkaloids of <i>Lobelia</i></b>	175
a. TLC of <i>Lobelia</i> alkloids	175
b. HPLC of <i>Lobelia</i> alkaloids	176
c. Estimation of total alkaloids –Titrimetry	176
<b>6.6.5. Alkaloids of papaya</b>	176
a. TLC of papaya alkaloids	177
b. HPLC of carpaine	177
c. Isolation of carpaine and pseudocarpaine	177
<b>6.6.6. Alkaloids of pomegranate</b>	178
a. TLC of pomegranate alkaloids	178
b. HPLC of pomegranate alkaloids	178
c. Isolation of alkaloids	178
d. Tests for pomegranate alkaloids	179
e. Estimation of pomegranate alkaloids – Titrimetry	179
<b>6.7. Quinazoline alkaloids</b>	179
<b>6.7.1. Alkaloids of <i>Adhatoda</i></b>	179
a. TLC of <i>Adhatoda</i> alkaloids	180
b. HPLC of <i>Adhatoda</i> alkaloids	180
c. Estimation of total alkaloids	180
d. Isolation of vasicine and vasicinone	181
<b>6.8. Quinoline alkaloids</b>	181
<b>6.8.1. Alkaloids of <i>Cinchona</i></b>	181
a. Extraction of alkaloids	182
b. Tests for quinine	182
c. PC of <i>Cinchona</i> alkaloids	182
d. TLC of <i>Cinchona</i> alkaloids	183
e. HPLC of <i>Cinchona</i> alkaloids	183
f. Estimation of total alkaloids – Gravimetry	183
<b>6.9. Steroidal alkaloids</b>	184
<b>6.9.1. Alkaloids of <i>Holarrhena</i></b>	184
a. PC/TLC of <i>Holarrhena</i> alkaloids	184

b. HPLC of <i>Holarrhena</i> alkaloids	184
c. Estimation of total alkaloids- Gravimetry	185
d. Isolation of conessine from seeds	185
e. Tests for conessine	185
<b>6.9.2. Alkaloids of <i>Solanum</i></b>	186
a. TLC of <i>Solanum</i> alkaloids	186
b. HPLC of <i>Solanum</i> alkaloids	186
c. Isolation of solanine from Potato	186
d. Tests for alkaloids of <i>Solanum</i>	187
e. Estimation of solanine and solanidine – Spectrophotometry	187
f. Extraction of solasodine from <i>Solanum khasianum</i>	187
<b>6.10. Tropane alkaloids</b>	188
a. Isolation of alkaloids	188
b. Tests for tropane alkaloids	189
c. TLC of tropane alkaloids	189
d. HPLC of tropane alkaloids	189
e. Estimation of solanaceous tropane alkaloids – Titrimetry	190
f. Estimation of coca alkaloids- Titrimetry	190
<b>6.11 Tropolone alkaloids</b>	191
<b>6.11.1. Alkaloids of <i>Colchicum</i></b>	191
a. TLC of <i>Colchicum</i> alkaloids	191
b. Tests for colchicines	191
c. HPLC of <i>Colchicum</i> alkaloids	191
d. Estimation of colchicines : Gravimetric method	192
<b>7. Phenolics</b>	<b>193-251</b>
<b>7.1. Simple phenols and benzoic acids</b>	193
a. Extraction of phenolic acids	196
b. Analysis of phenolic acids by 2-D PC	196
c. TLC of phenolic acids	198
d. HPLC of phenolic acids	199
<b>7.2. Phenylpropanoids</b>	199
<b>7.2.1. Cinnamic acids</b>	199
a. Isolation of chlorogenic acid from coffee	201
b. Tests for chlorogenic acid	202
c. PC and TLC of chlorogenic acid	202
d. TLC of chlorogenic acid	202
<b>7.2.2. Capsaicin from red chillies</b>	202

a. TLC of capsaicin	202
b. HPLC of capsaicin	203
7.2.3. <b>Curcuminoids from turmeric</b>	203
a. TLC of curcuminoids	203
b. HPLC of curcuminoids	203
7.2.4. <b>Coumarins</b>	204
a. Extraction of coumarins	205
a. TLC of coumarins	205
b. HPLC of coumarins	206
7.2.4.1. Coumarins of <i>Aegle</i>	206
a. Isolation of marmelosin from unripe fruits	207
b. Isolation of marmesin, marmin and umbelliferone (and $\beta$ -sitosterol) from the stem bark	207
7.2.4.2 Coumarins of <i>Psoralea</i>	207
a. TLC of coumarins of <i>Psoralea</i>	207
b. HPLC of coumarins of <i>Psoralea</i>	207
7.2.5. <b>Chromones</b>	208
1. Chromones of Visnaga	208
a. Extraction of khellin	208
b. PC of Visnaga chromones	208
c. HPLC of khellin	209
7.2.6. <b>Lignans</b>	209
a. Extraction of Lignans	211
1. <b>Lignans of <i>Phyllanthus amarus</i></b>	211
a. HPTLC analysis of <i>Phyllanthus</i> lignans	212
b. HPLC of <i>Phyllanthus</i> lignans	212
2. <b>Lignans of <i>Schizandra</i></b>	212
a. TLC of lignans of <i>Schizandra</i>	213
b HPLC of lignans of <i>Schizandra</i>	213
3. <b>Lignans of Mandrake</b>	213
a. TLC of Mandrake lignans	213
b. HPLC of Mandrake lignans	214
4. <b>Flax seed lignans</b>	214
a. Extraction of lignans	215
a. TLC of Flax seed lignans	215
b. HPLC of Flax seed lignans	215
5. <b>Sesame oil lignans</b>	216
a. Isolation of lignans by saponification	217

b. TLC of Sesame lignans	217
c. HPLC of Sesame lignans	217
d. Isolation of sesamol	217
<b>7.3. Flavonoids</b>	218
1. Flavones and flavonols	219
1. Analysis of flavones/flavonols	220
a. PC of flavonols/flavones	220
b. TLC of flavonols of tea	221
c. HPLC of flavones/flavonols	221
2. Isoflavones, isoflavonones and homoisoflavones	222
1. Soybean isoflavones	223
a. Extraction of soybean isoflavones	224
b. TLC of soybean isoflavones	224
c. HPLC of soybean isoflavones	224
3. Anthocyanins	224
1. Anthocyanins from flowers	225
a. Extraction and PC of anthocyanins	226
b. Extraction and PC of anthocyanidins	226
c. HPLC of anthocyanins	227
4. Chalcones	228
a. Butein from <i>Butea</i> flowers	228
b. Tests for butein	229
5. Neoflavonoids	229
1. Rottlerin from Kamala dye	229
6. Flavonones and flavononols	230
1. Flavonolignans from <i>Silybium</i>	231
a. TLC of <i>Silybium</i> flavonolignans	232
b. HPLC <i>Silybium</i> flavonolignans	232
c. Column chromatography of flavonolignans	232
2. Gossypol from cotton seed and <i>Thespesia</i> flowers	233
7. Flavan-3-ols, flavan-3, 4-diols and proanthocyanidins	234
a. Analysis of proanthocyanidins	234
8. Aurones	235
9. Biflavones	235
<b>7.4. Quinones</b>	<b>234-245</b>
7.4.1. Benzoquinones	236
1. Thymoquinone from <i>Nigella sativa</i> L. (Black Cumin)	236
a. Extraction of oil	236

b. TLC of thymoquinone	237
c. GC and GC-MS analysis of thymoquinone	237
d. HPLC of thymoquinone	237
2. <b>Embelin from <i>Embelia</i></b>	237
a. PC of embelin	237
b. TLC of embelin	238
c. HPLC of embelin	238
d. Estimation of embelin-titrimetry	238
2. <b>Naphthoquinones</b>	239
1. Quinones of <i>Lawsonia</i>	239
a. PC of lawsone	240
b. HPLC of lawsone	240
3. <b>Anthraquinones</b>	240
1. Quinones of <i>Aloe</i>	242
a. TLC of <i>Aloe</i> quinones	242
b. HPLC of <i>Aloe</i> quinones	243
2. Quinones of Senna	243
a. TLC of senna quinones	243
b. HPLC of senna quinones	243
3. Rhein from <i>Cassia fistula</i>	243
a. Isolation of rhein	244
b. TLC of quinones of rhein	244
3. Santalins from Red Sandal Wood	245
<b>7.5. Tannins</b>	<b>245-249</b>
a. Tests for tannins	247
b. Tests for hydrolysable tannins	247
c. Estimation of gallotannins – spectrophotometry	248
d. Estimation of total tannins – Indigocarmin method	248
e. Analysis of proanthocyanidins	249
<b>7.6. Lignin</b>	<b>249-251</b>
a. Tests for lignin	250
b. Estimation of lignin	250
c. Analysis of lignin in Angiosperms and Gymnosperms	251
<b>8. Other nitrogen containing compounds</b>	<b>252-255</b>
<b>8.1 Amines</b>	<b>252</b>
a. PC of volatile amines	252
b. HPLC of amines	252

<b>8.2. Cyanogenic glycosides</b>	252
a. PC of cyanogenic glycosides	253
b. TLC of cyanogenic glycosides	253
c. HPLC of cyanogenic glycosides	253
<b>8.3 Indigo from <i>Indigofera</i></b>	253
<b>8.4. Chlorophylls</b>	254
a. Chromatographic analysis of chlorophylls	254
b. Estimation of chlorophylls	255
<b>9. Sulphur containing compounds</b>	<b>256- 260</b>
<b>9.1 Glucosinolates</b>	256
a. Extraction of glucosinolates	257
b. PC of glucosinolates from mustard	257
c. HPLC of glucosinolates	258
<b>9.2 Sulphides</b>	258
a. Sulphides from garlic	258
<b>9.3 Thiophenes</b>	259
a. TLC of thiophenes	260
<b>10. Alkanes, Acetogenins and Polyacetylenes</b>	<b>261- 267</b>
<b>10.1 Alkanes</b>	261
a. Extraction of alkanes	262
b. TLC of alkanes	263
c. GC of alkanes	263
<b>10.2. Annonaceous acetogenins</b>	263
a. Extraction of acetogenins	265
b. TLC of acetogenins	265
c. HPLC of acetogenins	265
<b>10.3. Polyacetylenes</b>	265
a. Extraction of polyacetylenes	266
b. TLC of polyacetylenes	266
c. HPLC of polyacetylenes	267
<b>References</b>	<b>268- 281</b>
<b>Index</b>	<b>282-290</b>