Modern Phytomedicine

Turning Medicinal Plants into Drugs

Edited by
Iqbal Ahmad, Farrukh Aqil, and Mohammad Owais
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Modern Phytomedicine

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Contents

Preface XV

List of Contributors XVII

1 Bioactive Phytocompounds: New Approaches in the Phytosciences 1
Ricardo Ramos Mendonça-Filho
1.1 Introduction 2
1.2 Development of Fast Reliable Methods of Extraction and High-Throughoutput Screening (HTS) of Crude Plant Extracts: New Challenges 3
1.3 Antimicrobial Bioactive Phytocompounds from Extraction to Identification: Process Standardization 6
1.4 Problems Associated with the Efficacy, Stability and Quality Control of Herbal Drugs Preparations 13
1.5 Novel Bioactive Phytocompounds Against Multidrug-Resistant Bacteria/Fungi: The Management of Infectious and Chronic Diseases 17
1.6 Mode of Action of Bioactive Phytocompounds and their Interactions with Macromolecules and Toxicity 18
1.7 Bioactive Phytocompounds and Future Perspectives 21
References 23

2 Quality Control, Screening, Toxicity, and Regulation of Herbal Drugs 25
Wickramasinghe M. Bandaranayake
2.1 Introduction 26
2.2 Preparation of Herbal Drugs 29
2.3 Quality Control of Herbal Drugs 30
2.3.1 Parameters for Quality Control of Herbal Drugs 34
2.3.1.1 Microscopic Evaluation 34
2.3.1.2 Determination of Foreign Matter 34
2.3.1.3 Determination of Ash 35
2.3.1.4 Determination of Heavy Metals 35
4 Bioactive Phytocompounds and Products Traditionally Used in Japan

Jin-ichi Sasaki

4.1 Introduction 80
4.2 Garlic 80
4.2.1 Introduction 80
4.2.2 Biological Effect of Garlic 81
4.2.2.1 Antibacterial Effects 81
4.2.2.2 Anticoagulation Effects 84
4.2.2.3 Antioxidant Activity 86
4.2.2.4 Therapeutic Effects of Garlic Powder in the Organophosphate Compound Poisoning Mouse as a Model of SARS 87
4.3 Mushroom 87
4.3.1 Introduction 87
4.3.2 Biological Effects 88
4.3.2.1 Antitumor Activity 88
4.4 Sweetcorn 92
4.4.1 Introduction 92
4.4.2 Biological Effects 92
4.4.2.1 Antitumor Activity of Sweetcorn 92
4.5 Oil and Flavor of Tree Hiba (Japanese Cypress) (Hinokitiol) 94
4.5.1 Introduction 94
4.5.2 Biological Effects 94
4.6 Conclusions 95
Acknowledgments 96
References 96

5 Plant Extracts Used to Manage Bacterial, Fungal, and Parasitic Infections in Southern Africa

J.N. Eloff and L.J. McGaw

5.1 Introduction 98
5.2 Biodiversity in Southern Africa 99
5.3 Use of Plants in Southern African Traditional Medicine 99
5.4 The Need for Anti-Infective Agents 100
5.5 Selection of Plant Species to Investigate 100
5.5.1 Ethnobotanical Approach 101
5.5.2 Chemotaxonomy 101
5.5.3 Random Selection 101
5.6 Collecting, Drying, and Storage of Plant Material 102
5.7 Extraction of Plant Material 103
5.7.1 Which is the Best Extractant? 103
5.7.2 Extraction Period and Efficiency 104
5.7.3 Selective Extraction 104
5.7.4 Redissolving Extracts for Quantitative Data 105
5.7.5 Storage of Extracts 105
5.8 Evaluating Quantitative Antimicrobial Activity 105
7.2 Phytoalexins and Phytoncides 139
7.3 Antibiotics 140
7.4 Bacteria and Broth 140
7.4.1 VRE 140
7.4.2 VSE 141
7.4.3 MRSA 141
7.4.4 MSSA 141
7.4.5 Broth 141
7.5 Isolation of Phytoalexins and Phytoncides 141
7.6 Minimum Inhibitory Concentration 142
7.7 Synergism of Antibacterial Compounds with Commercially Available Antibiotics 142
7.8 Antibacterial Activities 143
7.8.1 Sophoraflavanone G 143
7.8.2 Calozeleyoxanthone 144
7.8.3 α-Mangostin 144
7.8.4 Gnemonol B and Gnetin E 145
7.8.5 Summary of MIC Values of Phytoalexin and Phytoncide Against MRSA and VRE 146
7.9 Synergism Between the Test Compounds and Commercial Antibiotics Against VRE, MRSA, VSE, and MSSA 147
7.9.1 Sophoraflavanone G 147
7.9.2 Calozeleyoxanthone 148
7.9.3 α-Mangostin 148
7.9.4 Stilbene Oligomer 151
7.9.5 Summary of Synergistic Effects Between the Test Compounds and the Commercial Antibiotics Against VRE and MRSA 153
References 154

8 Methods for Testing the Antimicrobial Activity of Extracts 157
Jenny M. Wilkinson
8.1 Introduction 157
8.2 Antibacterial Assays 158
8.2.1 Semi-Solid Substrate Methods 161
8.2.1.1 Disk Diffusion Method 161
8.2.1.2 Agar Dilution Method 162
8.2.1.3 Broth Dilution Methods 163
8.2.1.4 Thin-Layer Chromatography–Bioautography 164
8.3 Antifungal Assays 165
8.4 In vivo Assessment of Antibacterial and Antifungal Activity 166
8.5 Methods for Assessing Antiviral Activity 167
8.6 Screening of Plant Extracts for Antiparasitic Activity 167
8.7 Conclusions 168
References 169
9 Targeted Screening of Bioactive Plant Extracts and Phytocompounds Against Problematic Groups of Multidrug-Resistant Bacteria
Farrukh Aqil, Iqbal Ahmad, and Mohammad Owais

9.1 Introduction 174
9.1.1 Multiple Antibiotic Resistance in Bacteria 174
9.1.2 Plants as a Source of Novel Bioactive Compounds 177
9.2 Approaches to Targeted Screening Against MDR Bacteria 179
9.2.1 MDR Efflux Pump Inhibitors from Plants 180
9.2.2 β-Lactamase Inhibitors 181
9.2.3 Synergy Between Phytocompounds and Antibiotics 182
9.2.4 Targeting Virulence and Pathogenicity 185
9.2.5 Quorum Sensing Inhibitors 186
9.3 Other Potential Approaches 189
9.3.1 Targeting Gene Transfer Mechanisms 189
9.3.2 Targeting R-Plasmid Elimination 190
9.4 Conclusions and Future Directions 191
Acknowledgments 192
References 193

10 Activity of Plant Extracts and Plant-Derived Compounds against Drug-Resistant Microorganisms
Antonia Nostro

10.1 Introduction 199
10.2 Plant Materials with General Antimicrobial Activity Including some Drug-Resistant Strains 200
10.3 Plant Materials with Specific Antimicrobial Activity Against Drug-Resistant Strains 201
10.3.1 Drug-Resistant Gram-Positive Bacteria 201
10.3.2 Drug-Resistant Gram-Negative Bacteria 211
10.3.3 Other Drug-Resistant Microorganisms 212
10.4 Plant Materials that Restore the Effectiveness of Antimicrobial Agents and/or Inhibit Drug Resistance Mechanisms 223
10.4.1 Other Mechanisms 225
10.5 Conclusions 226
References 226

11 An Alternative Holistic Medicinal Approach to the Total Management of Hepatic Disorders: A Novel Polyherbal Formulation
Mohammad Owais, Iqbal Ahmad, Shazia Khan, Umber Khan, and Nadeem Ahmad

11.1 Introduction 233
11.2 Conventional Medicines for Liver Disorders 236
11.3 Herbal Medicines – Potential Therapeutic Agents with Minimal Side-Effects 237
11.4 Contributions of Elementology to Potential Treatments for Hepatic Disorders  240
11.5 Other Alternatives in Liver Therapy  242
11.6 Conclusions  242
References  243

12 Traditional Plants and Herbal Remedies Used in the Treatment of Diarrheal Disease: Mode of Action, Quality, Efficacy, and Safety Considerations  247
Enzo A. Palombo
12.1 Introduction  248
12.2 Methods Used in the Evaluation of Bioactivity of Medicinal Plants  249
12.2.1 Antibacterial Activity  249
12.2.2 Antiprotozoal Activity  250
12.2.3 Antihelminthic Activity  250
12.2.4 Antiviral Activity  250
12.2.5 Antidiarrheal Activity  251
12.3 Traditional Medicinal Plants Used in the Treatment of Diarrhea that Display Antimicrobial Activity  252
12.4 Traditional Medicinal Plants Used in the Treatment of Diarrhea that Display Antidiarrheal Activity  255
12.5 Phytochemical Analysis, Identification of Active Plant Components, and Mechanism of Action of Medicinal Plants Used in the Treatment of Diarrhea  260
12.6 Quality, Efficacy, and Safety Considerations  263
12.7 Conclusions  266
Acknowledgments  267
References  267

13 Mutagenicity and Antimutagenicity of Medicinal Plants  271
Javed Musarrat, Farrukh Aqil, and Iqbal Ahmad
13.1 Introduction  271
13.2 Plants as Protective Agents Against DNA Damage  272
13.3 Antimutagenic Properties of Edible and Medicinal Plants  274
13.4 Mutagenicity of Plant Extracts and Phytochemicals  279
13.5 “Janus Carcinogens and Mutagens”  280
13.6 Chemical Nature of Phytoantimutagenic Compounds  281
13.6.1 Flavonoids  282
13.6.2 Phenolic Compounds  282
13.6.3 Coumarins  282
13.6.4 Diterpenoids  282
13.6.5 Organosulfur Compounds  283
13.7 Assays for Mutagenicity and Antimutagenicity  283
13.8 Paradigms in Antimutagenicity Research  285
Contents

16 Immunomodulatory Effects of Phytocompounds 341
   Buket Cicioğlu Arıdoğan
   16.1 Introduction 342
   16.1.1 General Properties and Classification of Phytocompounds 342
   16.2 Effect of Specific Medicinal Herbs on Immune System and Immune Cells 343
   16.3 General Properties of Echinacea Species 344
   16.4 Effects of Echinacea Species on the Immune System and Various Immune Cells 345
   16.5 Asteraceae 349
   16.6 Lithospermum erythrorhizon 351
   16.7 Guarana 352
   16.8 Side and Adverse Effects of Some Phytocompounds 352
   16.9 Conclusion 353
   References 354

17 Use of a Liposomal Delivery System for Herbal-Based Therapeutics (with a Focus on Clove Oil) 357
   Nadeem Ahmad, Maroof Alam, Iqbal Ahmad, and Mohammad Owais
   17.1 Introduction 357
   17.1.1 Cinnamon Oil 359
   17.1.2 Oregano Oil 359
   17.1.3 Clove Oil 359
   17.1.3.1 Composition of the Clove Oil Used 360
   17.2 Rationale for Using Liposomized Formulation of Clove Oil 361
   17.2.1 Advantageous Properties of Liposomes 362
   17.3 Experiments Conducted to Develop Liposomal Clove Oil Formulation 362
   17.3.1 Determination of MIC of Clove Oil against Candida albicans 363
   17.3.2 Determination of MIC of Clove Oil against Escherichia coli 363
   17.3.3 In Vitro Antibacterial Activity Test Results 363
   17.3.4 In Vitro Antifungal Activity Tests Results (Table 17.4) 364
   17.3.5 In Vivo Antifungal Activity Test Results against Experimental Vaginal Candidiasis 364
   17.3.5.1 Evaluation of Efficacy of Liposomized Clove Oil 364
   17.3.5.2 Evaluation of Route of Administration 365
   17.4 Conclusions 366
   References 366

Subject Index 369
Medicinal preparations derived from natural sources, especially from plants, have been in widespread use since time immemorial. Ancient texts of India and China contain exhaustive depictions of the use of a variety of plant-derived medications. In fact, plants remain the main source of medicines for a large proportion of the world’s population, particularly in the developing world, despite the advent of the pharmaceutical chemistry during the early twentieth century, which brought with it the ability to synthesize an enormous variety of medicinal drug molecules and allowed the treatment of previously incurable and/or life-threatening diseases.

Not surprisingly, chemically synthesized drugs gained popularity and became the basis of pharmaceutical industry. Over the years, however, synthetic drugs have been plagued by unwanted side-effects, toxicity, and inefficiency, among other problems. In addition, the search for new drugs against a variety of illnesses through chemical synthesis and other modern approaches has not been encouraging. These factors, as well as the emergence of new infectious diseases, the proliferation of disorders such as cancer, and growing multidrug resistance in pathogenic microorganisms, have prompted renewed interest in the discovery of potential drug molecules from medicinal plants.

Herbal medicine is now globally accepted as a valid alternative system of therapy in the form of pharmaceuticals, functional foods, etc., a trend recognized and advocated by the World Health Organization (WHO). Various studies around the world, especially in Europe, have been initiated to develop scientific evidence-based rational herbal therapies. Though ancient medical treatises have documented a large number of medicinal plants, most have remained undocumented and uncharacterized, the knowledge of their use being passed down from generation to generation by word of mouth. New plant sources of medicine are also being discovered.

Here we have made an attempt to bring together recent work and current trends in the field of modern phytomedicine from different parts of the world. Although there are a number of books available on medicinal plants and phytocompounds, this book has unique contributions in the form of chapters from experts in the field starting from the concept of phytoscience, screening biological activities against problematic infectious agents such as multidrug-resistant bacteria, fungi, and viruses. Discussion of types of herbal remedies, problems associated with herbal
medicines, such as efficacy, adulteration, safety, toxicity, regulations, and drug delivery etc. are included as contributions by different learned experts.

This book is intended to cover recent trends in phytomedicine and future perspectives in human health care. It is intended that this book will be useful to students, teachers, and researchers in universities, R & D institutions, pharmaceutical and herbal industries as well as to health organizations.

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Bioactive Phytocompounds: New Approaches in the Phytosciences

Ricardo Ramos Mendonça-Filho

Summary

Today’s use of medicinal plants and bioactive phytocompounds worldwide and our scientific knowledge of them comprises the modern field of the “phytosciences.” The phytosciences have been created from the integration of disciplines that have never been linked before, combining diverse areas of economic, social, and political fields, chemistry, biochemistry, physiology, microbiology, medicine, and agriculture.

The field is unique among the biomedical sciences in that instead of testing a hypothesis, in the phytosciences researchers try to determine whether plants commonly used in traditional medicine brings benefits for health and, if so, what their mechanisms of action are.

Despite the common belief that phytocompounds are safe, they all have inherent risks just like synthetic compounds. Thus it is within the scope of the phytosciences to elucidate side-effects, appropriate doses, identify bioactive phytocompounds and ways of extraction and conservation. Besides these, legal aspects regarding regulation of the prescription and commercial sale of medicinal plants are a matter of debate all around the world. The varied regulations in different jurisdictions regarding the prescription and sale of these products add confusion to the formal use of phytocompounds.

As a multidisciplinary science, research in the phytosciences is almost unlimited, which makes it impossible to discuss all aspects of this emerging science in just one chapter. Therefore, we have focussed here mainly on the antimicrobial activity of bioactive phytocompounds, discussing their use against multidrug-resistant (MDR) bacteria and fungi, their mechanisms of action, and their interactions with macromolecules and potential for toxicity in mammalian cells. Technical aspects regarding the development of fast and reliable methods of extraction, high-output screening systems, and bioautography of essential oils and crude extracts and fractions have also been discussed. Problems related to the efficacy, stability, drug delivery systems and quality control are also commented on.

Overall this chapter aims to provide a better understanding of the modern field of the phytosciences and its application in the world today.
1.1 Introduction

To trace the history of phytotherapy is to trace the history of humanity itself. The discovery of the curative properties of certain plants must have sprung from instinct. Primitive peoples first used plants as food and, as result of this ingestion, the link with some plant properties would have been learnt. Medicinal plants were the main source of products used to sustain health until the nineteenth century, when the German chemist Friedrich Wöhler in 1828, attempting to prepare ammonium cyanate from silver cyanide and ammonium chloride, accidentally synthesized urea. This was the first organic synthesis in history and heralded the era of the synthetic compound.

Fig. 1.1 Pedanius Dioscorides, De Materia Medica (AD 65). Greek physician Pedanius Dioscorides (c. 40–c. 90) was from Anazarbus, a small town near Tarsus in what is now south-central Turkey. As a surgeon with the Roman army of Emperor Nero, Dioscorides traveled through Italy, Gaul, Spain, and North Africa, recording the existence and medicinal value of hundreds of plants. He compiled an extensive listing of medicinal herbs and their virtues in about AD 70. Originally written in Greek, Dioscorides’s herbal was later translated into Latin as De Materia Medica. It remained the authority in medicinal plants for over 1500 years.
During the 100 years following Wöhler’s discovery phytomedicine was largely forgotten by Western science. In the early 1980s, however, there was a resurgence of interest in the use of natural substances generally known today as bioactive phyto-compounds. This interest can be easily understood in the light of questions concerning the safety, cytotoxicity, and side-effects of synthetic compounds, and the need to find new medicines, including new antibiotics to manage infectious diseases caused by multiresistant pathogens and substances to treat chronic diseases.

Today, the use of medicinal plants and their bioactive phyto-compounds and our scientific knowledge about them comprises the modern field of the phytosciences. This is a science created from the integration of a range of disciplines that have never been linked before, combining several different areas of economic, social, and political fields, chemistry, biochemistry, physiology, microbiology, medicine, and agriculture.

The phytosciences are different from the other biomedical sciences in that instead of testing a hypothesis, researchers try to determine whether plants commonly used in traditional medicine bring benefits for health and, if so, what are their mechanisms of action. Despite the common belief that bioactive phyto-compounds are safe, they have inherent risks just like all active chemical compounds. Researchers within the phytosciences are working to elucidate the side-effects, calculate appropriate dosages, identify the bioactive components, and define the best methods of extraction and conservation. Besides these, legal aspects regarding the prescription and trade in medicinal plants are a matter of debate all around the world. The varying regulations in different jurisdictions allowing the prescription and sale of these products add confusion to the formal use of bioactive phyto-compounds.

As a multidisciplinary science the research in this field is almost unlimited, which makes it impractical to discuss all the aspects of this emerging science in just one chapter. Therefore, this review discusses the antimicrobial activity of bioactive phyto-compounds, particularly their use against multidrug-resistant bacteria and fungi, their mechanisms of action, and their interactions with macromolecules and potential toxicity for mammalian cells. It also discusses technical aspects regarding the development of fast and reliable methods of extraction, high-output screening systems and bioauthography of essential oils and crude extracts and fractions. Problems related to the efficacy, stability, drug delivery systems and quality control will also be discussed.

1.2 Development of Fast Reliable Methods of Extraction and High-Throughput Screening (HTS) of Crude Plant Extracts: New Challenges

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in international trade. Recognition of their clinical, pharmaceutical, and economic value is still growing, although this varies widely between countries. Plants are important for pharmacological research and drug development, not on-
ly when bioactive phytocompounds are used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds. Regulation of their exploitation and exportation is therefore essential to ensure their availability for the future [1].

Plant preparations have a very special characteristic that distinguishes them from chemical drugs: a single plant may contain a great number of bioactive phytocompounds and a combination of plants even more. This complexity is one of the most important challenges to phytoscientists attempting to identify a single bioactive phytocompound or chemical group in the enormous universe that comprises a single crude extract.

Biotechnology in the 1970s and 1980s made tremendous strides and ushered in a new era for the pharmaceutical industry. Many enzymes and receptor proteins of therapeutic interest were made available in large quantities by recombinant expression, while signal transduction pathways could be interrogated by reporter gene carrying cellular constructs. Such mechanism-based *in vitro* assays are amenable to large scales of operation, and the concept of high-throughput screening rapidly became the paradigm for lead discovery [2].

High-throughput screening, often abbreviated as HTS, is a method of scientific experimentation especially relevant to the fields of biology and chemistry. Through a combination of modern robotics and other specialized laboratory hardware, it allows a researcher to effectively conduct hundreds of scientific experiments at once. In essence, HTS uses a brute-force approach to collect a large amount of experimental data, usually observations about how some biological entity reacts to exposure to various chemical compounds in a relatively short time. A screen, in this context, is the larger experiment, with a single goal to which all this data may subsequently be applied [3].

A necessary precondition for the success of the HTS approach is a large and diverse compound collection. In the early days, this largely comprised in-house archives and natural product extracts. The former represented the efforts of chemists internally over the years, supplemented by purchase from external sources. Neither the total number of compounds, nor their chemical diversity, was appropriate to feed HTS. These deficiencies created the science of combinatorial chemistry in the late 1980s and early 1990s and an unanticipated repercussion of high-throughput chemical synthesis was a steady waning of interest in natural product screening, leading to its complete abandonment by many companies [4].

Just like drugs of synthetic origin, bioactive phytocompounds range from simple to complex structures. Either way, the evaluation of a bioactive phytocompound or a natural product leads to benefits from modern HTS for the generation of analogs [5]. Thus, paradoxically, the same combinatorial chemistry that initially caused the decline in natural product screening now promises to be an essential tool in rejuvenating it. Academic groups in particular are used to allocating significant resources of time and staff towards the total synthesis of bioactive phytocompounds. The ability to adapt such routes for the preparation of analogs is an obvious strategy for leveraging the initial expenditure, and is now increasingly evident in the literature. Because of the stricter timelines, large-scale combinatorial programs
based on natural products are less common in industry, but are still practiced in the absence of more tractable synthetic leads [6].

Combinatorial chemistry has come a long way in the past two decades. Industrially, it competed with natural product extracts and purified bioactive phytocompounds for HTS resources and emerged as the preferred option. Unfortunately this technique has not produced a wealth of high-quality drug candidates. Instead, the integration of combinatorial chemistry with other mechanisms for lead generation is now rightly considered the correct strategy. A natural product lead is a legitimate starting point for combinatorial chemistry, and this process can often discover novel analogs [7]. In some cases, such compounds are more potent than the natural product or can possess superior drug-like properties. In others, the synthetic analogs display new biological activities not seen with the original molecule [4].

The ability to rapidly identify undesirable or desirable compounds in natural product extract libraries is a critical step in an efficiently run natural products discovery program. This process, commonly called dereplication [8], is important to prevent the unnecessary use of resources for the isolation of compounds of little or no value for development from extracts used in the screening process. Resources can then be focussed on samples containing the most promising leads. The recent application of HTS technologies to assay natural products extracts for biological activity has intensified the need for efficient dereplication strategies [9].

Dereplication of the bioactive phytocompounds in crude natural product extracts requires some form of feedback from the bioassay, which was initially used to detect the biological activity. This is necessary regardless of the separation technique and analytical method used. A common strategy has been to collect fractions from the high-performance liquid chromatography (HPLC) separation in deep-dish microtiter plates or tubes and then resubmit the individual fractions to the original assay. This approach requires desiccation of fractions to remove the HPLC solvents, which are usually incompatible with the bioassay, resuspending the fractions in a compatible solvent (water, DMSO, or Tween), and then individual assaying of each fraction. This process is not cost effective, being both time and labor intensive. Consequently, as a result of the increasing emphasis on the generation of new lead compounds, faster cycle times, and high efficiency, many pharmaceutical companies have moved away from the natural products area.

Currently, almost every large pharmaceutical company has established HTS infrastructures and possesses large combinatorial compound libraries, which cover a wide range of chemical diversity. However, the ability to detect the desired biological activity directly in the HPLC effluent stream and to chemically characterize the bioactive phytocompound on-line, would eliminate much of the time and labor taken in the fraction collection strategy. This way, cycle times, expenses, and the isolation of known or undesirable compounds would be reduced dramatically, allowing natural products to be screened in an efficient and cost effective manner [10].

Recently, such an on-line HPLC biochemical detection (BCD) system, in the following referred to as high-resolution screening (HRS) system, has been described for a range of pharmacologically relevant targets, such as the human estrogen receptor, cytokines, leukotrienes, and the urokinase receptor [11]. In contrast to con-
ventional microtiter-type bioassays, the interactions of the extracts and the biochemical reagents proceed at high speed in a closed continuous flow reaction detection system. When sufficient chromatographic separation is achieved, the individual contribution of the bioactive phytocompounds to the total bioactivity is obtained within a single run. Moreover, by combining on-line biochemical detection with complementary chemical analysis techniques, such as mass spectrometry (HRS-MS), chemical information that is crucial for the characterization and identification of bioactive phytocompounds is obtained in real time. Biochemical responses are rapidly correlated to the recorded MS and MS/MS data, thus providing chemical information such as molecular weight and MS/MS fingerprints [12]. Compared with traditional screening approaches of complex mixtures, which are often characterized by a repeating cycle of HPLC fractionation and biological screening, HRS-MS analysis speeds up the dereplication process dramatically. Moreover, the technology enables drug discovery programs to access the enormous chemical diversity offered by complex mixtures as a source of novel drug-like molecules [13]. The use of chromatographical assays is discussed in the next section of this chapter.

1.3 Antimicrobial Bioactive Phytocompounds from Extraction to Identification: Process Standardization

Different approaches to drug discovery using higher plants can be distinguished: random selection followed by chemical screening; random selection followed by one or more biological assays; biological activity reports and ethnomedical use of plants [14]. The latter approach includes plants used in traditional medical systems; herbalism, folklore, and shamanism; and the use of databases. The objective is the targeted isolation of new bioactive phytocompounds. When an active extract has been identified, the first task to be taken is the identification of the bioactive phytocompounds, and this can mean either a full identification of a bioactive phytocompound after purification or partial identification to the level of a family of known compounds [15].

In Fig. 1.2 an extraction-to-identification flowchart is proposed in order to optimize bioactive phytocompound identification. For screening selection, plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Initial screening of plants for possible antimicrobial activities typically begins by using crude aqueous or alcohol extractions followed by various organic extraction methods [16]. Plant material can be used fresh or dried. The aspects of plant collection and identification will be discussed further in this chapter. Other relevant plant materials related to antimicrobial activity are the essential oils. Essential oils are complex natural mixtures of volatile secondary metabolites, isolated from plants by hydro or steam distillation and by expression (citrus peel oils). The main constituents of essential oils (mono and sesquiterpenes), along with carbohydrates, alcohols, ethers, aldehydes, and ke-
tones, are responsible for the fragrant and biological properties of aromatic and medicinal plants. Due to these properties, since ancient times spices and herbs have been added to food, not only as flavoring agents but also as preservatives. For centuries essential oils have been isolated from different parts of plants and are also used for similar purposes.

The activities of essential oils cover a broad spectrum. Various essential oils produce pharmacological effects, demonstrating anti-inflammatory, antioxidant, and anticancerogenic properties [17–19]. Others are biocides against a broad range of organisms such as bacteria, fungi, protozoa, insects, plants, and viruses [20–22].

The dispersion of the hydrophobic components of essential oils in the growth medium is the main problem in testing the activity of essential oils. Different organic solvents must be used as solubilizing agents, which may interfere with the results of antimicrobial assays. The solution to this problem is the use of nonionic emulsifiers, such as Tween 20 and Tween 80. These molecules are relatively inactive and are widely applied as emulsifying agents. Control tests must guarantee that these emulsifying agents do not interfere in the experiments.

Plants can be dried in a number of ways: in the open air (shaded from direct sunlight); placed in thin layers on drying frames, wire-screened rooms, or in buildings; by direct sunlight, if appropriate; in drying ovens/rooms and solar dryers; by indirect fire; baking; lyophilization; microwave; or infrared devices. Where possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents. The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials. For example, shade drying is preferred to maintain or minimize loss of color of leaves and flowers; and lower temperatures should be employed in the case of medicinal plant materials containing volatile substances [23]. The drying conditions should be recorded. In the case of natural drying in the open air, medicinal plant materials should be spread out in thin layers on drying frames and stirred or turned frequently. In order to secure adequate air circulation, the drying frames should be located at a sufficient height above the ground. Efforts should be made to achieve uniform drying of medicinal plant materials to avoid mold formation [24].

Drying medicinal plant material directly on bare ground should be avoided. If a concrete or cement surface is used, the plant materials should be laid on a tarpaulin or other appropriate cloth or sheeting. Insects, rodents, birds and other pests, and livestock and domestic animals should be kept away from drying sites. For indoor drying, the duration of drying, drying temperature, humidity and other conditions should be determined on the basis of the plant part concerned (root, leaf, stem, bark, flower, etc.) and any volatile natural constituents, such as essential oils. If possible, the source of heat for direct drying (fire) should be limited to butane, propane or natural gas, and temperatures should be kept below 60 °C [25]. If other sources of fire are used, contact between those materials, smoke, and the medicinal plant material should be avoided.

Since researches are trying to identify bioactive phytocompounds in medicinal plant extracts generally used by local population to treat diseases and based on empiric knowledge that they have the searched bioactivity, the solvent chosen must be