Clinical Pharmacognosy Series

HERBAL MEDICINES

Development and Validation of Plant-Derived Medicines for Human Health

Edited by

Giacinto Bagetta • Marco Cosentino
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CRC Press
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Contents

Series Preface

A number of books dealing with herbal medicines are available on the shelf of any bookstore. Some of these have become reference books for students and professionals active in the fields of pharmaceutical botany, pharmacognosy, pharmacy, and phytotherapy. In keeping with the cultural backgrounds of the authors, the organization of these books varies in perspective. In most instances however, they are set in a general section incorporating, among other information, the taxonomic organization of medicinal plants and ethnopharmacological information (where available), followed by a section describing the pharmacological actions and therapeutic applications of herbal products. In some instances, books dealing first with the need (the disease and its treatment) and then with the herbal remedy can also be found.

So far, however, no book has provided a comprehensive view of the development process of herbal medicines from breeding and cultivation, through analytical, pharmacotoxicological, preclinical, and clinical issues to the epidemiology of herbal use.

Herbal Medicines: *Development and Validation of Plant-Derived Medicines for Human Health* has been conceived by the editors to provide a venue for individuals (undergraduate or postgraduate students, academic or industrial researchers) interested in the research and development of herbal medicines to translate the same into the clinic. In this respect, the book cannot be used as a reference text for any specific details of herbal products but only for an occasional description of individual products as a reference, organized knowledge in the whole chain of the development process. The book begins by describing the rationale followed by the European Legislator in defining the minimal requirements to be satisfied for a herbal medicine to be used and commercialized under an acceptable level of risk. The definition of the role and implementation of the Committee for Herbal Medicinal Products at the level of the European Medicines Agency completed the rationalization process for the use of herbal medicines. Such organization is reflected in the general organization set by the FDA with which the main objectives are shared. After almost a decade since the beginning of this regulatory process together with the accumulation, over the decades, of data from the scientific literature underlying the complexity of the conditions under which these products are used, the spontaneous request for increased knowledge on the efficacy and safety of "older" and "newer" products has ensued and is actually consolidated. Accordingly, rationalization of plant taxonomy and breeding, together with standardization of the contents of herbal components, has assumed a pivotal role in successful candidates for research and development. These aspects are dealt with in chapters incorporating complex biotechnological as well as analytical methodologies of fundamental importance for the whole process. However, the ethnopharmacological approach to the identification of products to be driven to development is not at all underestimated. Obviously, definition of the mode of action of phytocomplexes, in general, and its comparison versus single entities require, in particular, a great deal of effort from scientists also highly knowledgeable in the field of *in vitro* and *in vivo* models for their assessment. The book deals with aspects of randomized clinical trials, reporting methodologies and quality control studies for those herbal products of great clinical interest in areas where the unmet need is enormous and where complex interactions with conventional drugs demand the development of continuous surveillance of their use. A series of "case studies" illustrates the process of development of herbal medicines in practice. The reader is also provided with an appreciation of the dimension of the utilization of herbal medicines in Western countries and in South America. The contribution of herbal medicines to the rational basis for modern therapy completes a volume that starts with historical notes highlighting the importance of this area of research.

This book aims to be a reference for all those interested in the rational development of herbal drugs as pharmacotherapies, providing essential knowledge on the relevant issues and thus bridging the often existing gap between the different and heterogeneous disciplines required to ensure successful completion of the process, "from field, to bench and then to bedside."

The editors are indebted to Hilary Rowe whose talent made it possible for our project to come true. Also, we would like to thank all the people from Taylor & Francis for their highly qualified technical contribution and all our collaborators. A special thanks goes to Dr. Michelangelo Certo for collaborating during the final editing process of the book.

Gaetana Silvia Rigo
Giuseppe Ottavio Armocida

Preface

FROM CONVICTIONS IN ANCIENT SCIENCE TO PROBLEMS IN CONTEMPORARY SCIENCE

The name of Galen can certainly remind today's doctors and chemists of medical history; however, we doubt that they are as experts in "magistrali" remedies or Galenic medicine as the majority of their colleagues were 50 years ago. This is not the case of mere consonance of sounds which leads to the name of a doctor in ancient times back to the so-called Galenic prescriptions; it is rather the conscious coincidence of doctrines and philosophies and of teaching and belief typical of a very ancient and nonetheless still extremely actual practice.

Among the less young, there may be someone who still recollects artisan abilities, refined in small and humble laboratories equipped with albarellos, mortars, balances, and other instruments that made the chemist's profession irreplaceable and highly respected. This treasure of traditions has not been completely lost in the course of time, but tends to enrich museums of antiquarian furnishings, rather than to complete the operativeness of the various chemists' shops in our towns. It is not surprising that the most common prescriptions, which used to be the main task of any chemist, are today almost completely replaced by a very flourishing form of official pharmacopoeia; maybe more problematic is the fact that medicines for rare diseases, which, in part, depended more than others on the personal characteristics of the chemist and of his shop, often remain excluded, or even "orphans."

We know that the abovementioned situations can be dealt with by means of pharmacovigilance, unfortunately sometimes even meant as vigilance of pharmaceutical expense and not just on the safety of medicinal products. These terms can be applied appropriately, since in phytotherapy as well criteria of phytovigilance and evidence of effectiveness can be used, taking into consideration the requisites of controls and the regulatory issues of the clinic, which are different from those in basic research.

Regulatory guidelines, as we know them nowadays, had the merit to stimulate and accelerate the rationalization of phytotherapic preparations, without being prejudicial to the genuineness of a way of thinking that, as a whole, is based both on the analogy and at the same time on the difference between food and medicine. Food integrators prepared on a vegetable basis and plant-derived medicines both answer the wish to reapproach nature, in particular as *vis medicatrix naturae*. They have different origins, different productive processes, and various contexts of application. Both, however, represent what at first seems to be approachable by everyone, (mis)perceived innocuous, and possibly usable on the basis of an innate critical sense given by "nature." In this prospect, one cannot underestimate the problems and values of self-medication which have always nourished a current of thinking, both formative and practical, with anthropological hints as well, at the crossroads of popular and official medicine, the latter commonly considered more scholarly.

Even among the experts of the herbarium of officinal herbs, several positions exist, not opposite, neither complementary, possibly alternative: neuropaths, herborists, homeopaths, and others. This scenario still seems to leave a good margin to individual initiative, which, on the one side, may under-evaluate the risks of a medicative substance, but on the other side underscores the virtues of a kind of medicine that discovers local therapies for typically local disease, as clearly pointed out by the principles of ethnic medicine. Actually, pharmacologic studies, chemistry, and clinic evaluation are at the crossroad of ethnic pharmacology and medical use of substances of plant origin. In this context, models employed to represent health and disease emerge, which belong to a maybe less authoritative but also less depersonalized sphere, with respect to the one proposed by public medicine. The very language of botanic knowledge suggests partially symbolic interpretations of the botanical universe, opening a global vision well beyond the world of nature, by means of an imaginative key. In particular, in the area of phytotherapy, clear fractures between preventive and curative medicine do not appear. The inclination to the preventive approach, by means of nonfactual considerations resulting from accurate epidemiologic studies, shows a higher probability of success as it concentrates on the multifactorial aspect of disease, and therefore not only on the therapeutic properties of a single herb, but also on the potentialities of the phytocomplex. This is how science and cultural religious and nonreligious traditions meet, thus re-establishing a sort of balance among different biological systems.

A phytotherapeutic preparation, unlike a medicine, has often changed the forms of its contents in the course of time, without necessarily referring to concepts of hidden energies, benign or malignant magics. However, we know various elements connecting the ranks of empiricism and science. The evolution of medicine, the widening of its field of pertinence, and the enrichment of the instruments of research and intervention altogether have promoted diagnostic and therapeutic categories based on the progress of biomedicine, posing a hard challenge to those who do not want to leave the ancient therapeutic knowledge, which is still effective.

The experts who, in this book, will be discussing the applications of phytotherapy, base their operativeness on a good knowledge of the historical vision. It is therefore needless to mention ancient scientific events, from the first masters to the present course of swinging fortunes in the therapeutic use of herbs. Experimental pharmacology has raised medicine from the stagnation of empiricism; it has, at last, offered clinics extremely powerful and effective instruments, greatly changing the capacity of treating and curing. In spite of that, while progress is looked at with pride, other facts are very surprising. In the last decade, in big towns and later in smaller centers as well, besides chemists' shops, well supplied with scientifically attractive products, several inviting herbalists' shops have appeared, which, following knowledge not always as convincing as the knowledge of official medicine, offer natural remedies prepared with various herbs and made in different ways known by tradition. Clients stand uncertain outside the two shops, being frequently unable to choose between the genuine and simple appeal of herbs and the product of the pharmaceutical industry. The regulation of medicine used to be entrusted to a restricted circle of learned people, who were well versed in the treatises by Dioscorides. At the same time, in the absence of teaching by experts, nature used to combine with the wise practice of the observation of man, inspired by good judgment and confirmed by the precepts coming from experience, in a sort of union which is not devoid of validity and effectiveness.

It is time that the name of Aesculapius itself, the god of medicine, finds an etymologic basin in "Aex Heyl hopa," which in the Schythian language means "the hope of health lies in the woods," the current rules of the Schola Salernitana and the medieval Christian one had grown on the Greek-human tradition, renewed by the contribution of Jewish and Arab culture. The texts dealing with the virtues of the plants useful for both the preservation and the restoration of health have been quite numerous in the course of centuries, and the study of the simple has constituted the most important outline of the knowledge of medicine for centuries. In the splendor of a *Hortus Sanitatis* and in the well-illustrated editions by Pietro Mattioli, medicinal plants and their habitat were listed, described, and represented. The coming of the movable type of printing greatly widened the diffusion of learned culture in the knowledge of the simple, as a prelude to the so-called "botanic renaissance." The accuracy and detail of the original ohawing and the ability to carve a wooden block or engrave metal plate to multiply an illustration, even highly elaborate ones, in a large number of copies acquired great importance. For each plant, the useful parts and the exact harvest time, the methods of preparation of the medicine, and the effective amounts were described together with a list of specific properties and curative virtues besides the diseases that could be relieved. But several well-known examples of private books of prescriptions, between the Middle Ages and Modern Age, show that sometimes doctors did not feel satisfied with the teaching inherited from old books. They trusted suggestions from the artifices of alchemy and enriched them with "medicinal secrets." For this reason, several doctors compiled catalogs of indigenous herbs, susceptible to continuous revision and updating.

Late in the sixteenth century, when voyages to unknown distant lands became more frequent, among the many goods imported, plants never seen before reached Europe and naturalists had new and various panoramas in front of them. In addition to the use of "teriaca," the authoritative panacea, the list of specific remedies for each illness increased. Doctors and chemists, preserving ancient knowledge thanks to constant practice and exercise, practiced an empiric method which remained at the basis of their work for a long time. The amount of information increased enormously, and at the beginning of scientific renewal in the nineteenth century, organization of available information became unavoidable. Chemistry started to be interesting in the different aspects of herbs and the study of same, casting off all trammels. In this context, however, it is not necessary to go over well-known facts of the past again.

We previously commented on a "*Rutilio*," a manuscript of popular remedies by a "masciano," a name used to qualify a healer in the Puglia region in the South of Italy. The old exercise book, written by a scarcely learned hand, contained hundreds of remedies that, at first, seemed to originate from local rural tradition, while, after careful examination they proved to have been taken from a learned eighteenth-century book by Nicolas Lemery. Although there has sometimes been a passage from official science to the popular world, the opposite course has been more constant and useful, as it nourished the practice of medicine as well as scientific texts of botany and medical subjects. Anthropologists still suggest not disregarding the traditions of popular medicine all over the world. They maintain that research in our laboratories could be shorter if we carefully looked about in ethnomedicine. A bright example in this direction can be found in recent medical history. In 1967, Antonio Scarpa (1903–2000), a paediatrician of rare learning, founded the Italian Institute of ethnoiatry at the hospital of Varese, Italy, collecting the experiences of a long life of explorations and research on European and non-European traditional medicine. In African deserts, he studied the phenomenon of *lactatio agravidica* or *lactatio serotina*, which was unknown here. He noticed that in those areas, lacking in cattle mill, the production of milk in aged women, who had long passed fertility, was induced by means of chewing a kind of herb which was easily found there. Antonio Scarpa understood that nature, if correctly interpreted, offers medicine useful resources for its needs, and in the course of time various confirmations to his interesting experiences followed. As an itinerant doctor, Scarpa was curious about different lifestyles and always concentrated on the multitude of their anthropological reflexes. Anthropologists, who in an island of the Asian southeast wondered about the strange capacities of a tribe to cure mental disease, realized that people drank water from a well which was "contaminated" by highly concentrated contents of lithium. Medicine then was not yet ready to understand the meaning of the fact and only later on did technical and intellectual instruments demonstrate the utility of lithium salts to cure some psychiatric syndromes, dysthymia in particular.

Between the end of the eighteenth century and the beginning of the nineteenth century, a large number of researchers in northwestern Italy drew up a as complete as possible herbaria. The 800 pages or so of *Flora Medica* written by municipal doctor Gilberto Scotti and published in 1872 is one of the best examples. Gilberto Scotti could rely on bulky works by his famous predecessors and he certainly knew *Deliciae florae et faunae insubricae* (Pavia 1786–1788), by Giovanni Antonio Scopoli. He was certainly well acquainted with Giuseppe Comolli, too, a provincial doctor of Como, a correspondent member of the Medical-Botanic Society of London and the author of a treatise in seven volumes called *Flora comense disposta secondo il sistema di Linneo a comodo dei medici, degli speziali e dei dilettanti nelle escursioni botaniche* (Como e Pavia 1834–1847), which classified plants to instruct young doctors and chemists. He dedicated his book to his colleagues as well, to offer them some interesting hints for their activity. At the same time, thanks to his long practice as a parish doctor caring for the difficulties of poor people, he strove to offer medical assistance, to the have-nots, to help people who could not afford expensive medicines. He counted 72 families of herbs typical of the province of Como, northwestern Italy, subdivided into more than 400 species, which were named in Latin and vernacular, followed by a precise description of the plant and a short story of its qualities, along with its medical properties. It is an orderly list of both spontaneous herbs which grew along roads or streams and herbs which were grown in orchards or gardens for food or as ornaments. He used to collect each part of a plant: roots, bark, buds, leaves, flowers, seeds, and fruit. Medical substances could be either ready for use or were to be processed to obtain preparations to be given externally or internally such as oil, ointment, liniment, decoction, juice, eye drops, infusion, tincture, balm, serif, and fills. Scotti was guided by a curative tradition consolidated in the course of centuries of popular experience, not by an experimental laboratory. In the same years, Paolo Minonzio wrote about his own experience as a communal doctor in a small village near Varese. Poor farmers could not afford using his prescriptions, but were nevertheless cured "taking a bit longer, without resorting to the chemist's or using my prescriptions because of poverty." They used the herbs they easily found in their fields without spending any money: marsh, mallow, dog's tooth, woody nightshade, verbasco, rue, sage, elder, wormwood, white marrabio, poppy, and camomile. According to the doctor, all of them had "special, undoubted and quite beneficent medical virtues." Built according to traditional knowledge, the large Alpinia garden near Stresa collected old books of botany for its library more than 50 years ago, and there is still a lot of scientific interest nowadays.

Medicine has always had certainties that cannot be the truth, but from the various ancient sciences we have moved to the problems of contemporary science. Nowadays, a man educated in biomedicine tends to be suspicious of fields of culture which are not ruled by his experimental instruments and very often does not show any interest in any phenomenon or in any therapeutic practice, the effectiveness of which cannot be explained by means of an experimental method. But while a scientist is based on such a way of thinking, a common man is more open and confident. If phytotherapy has not yet been proved useful, as some scientists claim, it does not mean it is not useful at all. The consolidated knowledge of physiology and pathology is not yet able to explain several of the still existing mysteries about a healthy and an ill body, and this is why we must respect offers from different fields.

A sick person provided with overall generic medical–scientific knowledge proposed in elegant ways by mass media and easily achievable takes the place of both a doctor and a chemist during diagnostic and therapeutic activities. The well-known esteem and confidence once solemnly put in these figures have disappeared, as patients are very frequently confronting or even opposing them. One should perhaps teach respect of the reciprocal roles: if a doctor is required to be competent, a patient should be more confident.

Nowadays, we need doctors able to widen their learning beyond the rigor of experimental science toward a form of knowledge that is said to be "irregular," but is acknowledged to be useful.

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1 The European Legislation (Directive 2004/24/EC) Brings Clarification and Recognition to Herbal Medicinal Products

Giuseppe Nisticò and Dagmar Roth-Behrendt

CONTENTS

1.1 INTRODUCTION

The first use of medicinal herbs is lost in the mist of time. Man has always aspired to maintain or achieve a state of psychosomatic wellbeing and has therefore used naturally occurring substances of vegetables, animals, or mineral origin in order to alleviate pain and suffering or to obtain therapeutic effects in the case of illness.

While acknowledging the important role of modern pharmacology and synthetic drugs in controlling major diseases, we consider that even now there is a need and a place for medicinal herbs in medicine and as such, a better exploitation of their therapeutic potential is necessary. In fact, unlike a single active principle in synthetic drugs, one or more active principles are present in medicinal herbs or plants and they may interact favorably since they can have synergic effects which are not yet fully recognized.

Medicinal herbs may be administered to human beings in various ways and in a variety of pharmaceutical preparations (extracts, decoctions, tinctures, syrups, eyewashes, etc.). As with synthetic drugs, in order to obtain therapeutic effects and avoid harmful effects, it is of fundamental importance to use a range of the so-called "therapeutic doses." When we increase the doses of drugs and medicinal herbs, they first become toxic and ultimately lethal. The range between therapeutic and toxic doses is called "therapeutic window." If the therapeutic window is very narrow and if, as the doses are gradually increased, there is an immediate transition from therapeutic to toxic doses, we, in this case, define the substances as poisons. There are, therefore, drugs or herbs with a very high intrinsic potency which, if doses are increased slightly, can have toxic or lethal effects. Hence, it is essential to classify medicinal herbs based on the potency of the pharmacological activities and the toxicity of the active principles contained within them. In practice, while recognizing the value of treatment with traditional herbs, we should bear in mind that improper use can produce serious toxic effects.

Because of the increasingly pervasive subculture in modern society, according to which what is "natural" is good, every year, huge damage is caused by the inappropriate use of the so-called natural products. Recently, this was pointed out by the US Food and Drug Administration (FDA), which noted that there is a widespread abuse of the so-called dietary supplement in the United States.

In fact, approximately 123 million people use these products for various purposes, to combat obesity, to prevent cancer, to ease pain, to enhance sexual performance, to stimulate mood, concentration, and memory, to enhance immune responses, to increase muscle mass and physical performance, and so on.

So far, the FDA has registered about 2900 cases of toxic effects, including 104 deaths caused primarily by the abuse of *Ephedra*. According to the FDA, there is no quality control for the marketing of such herbs and most of them are adulterated by contamination (with herbicides, pesticides, heavy metals, dioxin, biphenyls, etc.). In the United States, the most widely used products are those based on ephedrine (*Ephedra sinica*), for which more than 1200 cases of toxic effects, including 70 deaths, have been reported.

The UK Government and the Committee on Drug Safety recently expressed deep concern about the low quality and safety standards of a number of medicinal herbs not registered as medicines in Great Britain, especially certain "ethnic medicinal herbs." In particular, a toxic ingredient of *Aristolochia*, now banned, was found in traditional Chinese medicinal products, probably as a result of accidental or voluntary substitution. This plant contains a number of active principles (aristolochic acids) which are carcinogenic and capable of inducing nephrotoxicity, leading to renal insufficiency, as observed in some patients in Great Britain. Furthermore, the agency found toxic levels of arsenic and mercury in a number of traditional Chinese medicinal herbs, and it is worried about the illegal adulteration of creams produced from Chinese medicinal herbs containing steroids. Toxic effects, pharmaco-intolerance (allergies and idiosyncrasies) caused by medicinal herbs, and their interactions with other drugs are described in detail in Ref [1].

Against this background, it was logical for the European Union to establish a new legislation to safeguard the health of consumers by, on the one hand, setting high quality and safety standards for medicinal herbs and, on the other hand, stipulating the inclusion, on labeling and in package leaflets, of scientifically verified, simple, and clear information including the potential toxicity of medicinal herbs and possible interaction with food products and/or other medicines taken simultaneously. All of these were done as it is essential, while acknowledging the value and therapeutic usefulness of medicinal herbs, to both step up scientific research and proceed with caution in administering them.

1.2 KEY POINTS OF THE LEGISLATION

The main points of the Directive are the following.

1. Ensure high-quality standards for traditional herbal medicinal products.
2. Take into account sufficient long-term use and experience in proof of efficacy.
3. Labeling and leaflets adjusted to herbal medicinal products.
4. Establishment of a new committee at the European Medicines Agency (EMA) level on Herbal Medicinal Products (HMPC).

In particular, in order to recognize a product as a traditional herbal medicine, a *simplified registration* procedure has been established fulfilling the following criteria.

1. The product should have indications exclusively appropriate for traditional herbal medicinal products that are intended for use without the supervision of a medical practitioner.
2. It is exclusively for administration in accordance with a specified strength and posology.
3. It is an oral, external, and/or inhalation preparation.
4. A certain period of traditional use is required (see below).
5. The data on its traditional use should be sufficient to prove it in the specified conditions to be safe and efficacious on the basis of long-lasting use and experience.

Concerning the *composition* of a traditional herbal medicine, the European Parliament advocated possibilities of a range of combinations. In fact, it is possible to add in the traditional herbal products vitamins or minerals, provided that their action is ancillary to that of the herbal active ingredients regarding the specified claimed indications.

Applicants should provide documents that show a *high-quality standard* of their products and any authorization or registration obtained in another Member State or in a third country to place the medicinal product on the market. A bibliographical or expert evidence is necessary to demonstrate that the medicinal product in question has been used throughout a *period of at least 30 years* preceding the date of the application, including at least 15 years within the EU. At the request of the Member State, the HMPC at the EMA shall draw an opinion on the adequacy of the evidence of long-lasting use of the product or of a corresponding product. Applicants should also provide a bibliographic review of *safety data* together with an expert report and where required by the competent authority, data necessary for assessing the safety of the medicinal product.

When the product has been in use in the community for less than 15 years, the Member State where the application for traditional-use registration has been submitted shall refer the product to the European HMPC.

If the committee considers it possible, it shall establish a community *herbal monograph* which shall be taken into account by the Member State for its final decision.

One of the most important achievements of the European Parliament to improve the traditional herbal medicines Directive was the establishment of the HMPC at the EMA. The role of this Committee involves the following.

1. Performing the tasks required to ascertain the quality, the composition, and the traditional use of the product.
2. Preparing Community herbal monographs which allow for the authorization of herbal medicinal products.
3. Preparing a list of herbal substances, preparations, and combinations.

The Committee consists of one member and one alternate appointed by each Member State chosen for their role and experience in the evaluation of herbal medicinal products. The Committee may also co-opt a maximum of five additional members chosen on the basis of their specific scientific competence.

The final text of the Directive gave to the Committee a wider scope of tasks including, in particular, the final decision in an *arbitration* process in cases where a mutual recognition procedure between EU Member States could not be finalized successfully. This enlarged the responsibility of the Committee and will ensure an appropriate assessment, giving confidence to the manufacturers in the area to submit applications as they can now expect to get the best available expertise in judging their products.

The new HMPC will have to have autonomy in decision-making and tasks from those envisaged for the Committee on Human Medicinal Products in Directive 2001/83/EC. Another aspect considered in the Directive is the obligation to include in *package leaflets* and other forms of publicity information to the effect that the therapeutic efficacy of traditional medicinal herbs has not been proven in specific clinical trials, as required by Directive 2001/83/EC.

Finally, the Directive lays down a harmonized legislative framework for all EU countries, promoting the free movement of traditional medicinal herbs in the community. Furthermore, the Directive guarantees the highest level of protection for public health on the basis of the documentation required concerning the quality, efficacy, and safety.

In September 2008, the European Commission published its report on the experience acquired as a result of the application of the provisions of Chapter 2a of Directive 2001/83/EC, as amended by Directive 2004/24/EC, on specific provisions applicable to traditional herbal medicinal products. The Commission's report admittedly has the merit of stressing clearly the fundamental objectives of Directive 2001/83/EC, which indicate that any medicinal product, before being authorized for sale, must be accompanied by a technical scientific dossier, documenting its quality, efficacy, and safety.

1.3 CONCLUSIONS

The approval of the Directive 2004/24/EC on traditional herbal medicinal products brings recognition to these important compounds as medicines. Nowadays, the progress achieved in the last decades with the use of new advanced therapies (biotechnological products, therapeutic use of stem cells, and gene therapy) will add a new dimension to the therapeutic armamentarium. However, we are conscious that no one can reproduce in laboratory all the complex biotechnological products present in nature (plants and herbals), which besides a natural balance of different active principles will certainly have a safer toxicological profile in comparison to monoclonal antibodies and other biotechnological and cell products.

The approval of the Directive came after more than 10 years of conflicting views and very intense and controversial discussions among the Commission, European Parliament, and all the other stakeholders. It was an honor and privilege for the authors of this chapter to co-ordinate the opinion of the European Parliament and to combine the specific scientific competence, one being professor in Pharmacology and Toxicology at the University of Rome Tor Vergata and the other with a specific and long-lasting professional competence on European legislation. This allowed us to reach a consensus among all the interested parties after the agreement between the Commission and the European Parliament on many amendments through compromise.

We are less satisfied with the slow progress in preparing the relative monographs by the European HMPC and particularly with the reluctance of some national authorities to take fully into account the monographs in the context of national applications.

In addition, we believe the progress which EMA is achieving in the collaboration with the European Food Safety Authority (Parma) is important in order to rapidly classify an herbal product as a medicinal product or instead as food.

We also believe that a new piece of legislation should deal with those traditional herbals not recognized as such within the 7 years after entry into force of the Directive as well as with other aspects such as extension of traditional-use registration to other categories of medicinal products or to the combinations of traditional herbals with amino acids, fatty acids, and so on.

The possibility will remain for herbals to be used in food stuff and as food supplements depending on the national legislation in the EU Member States which may be harmonized in the future by an amendment of the EU food supplement Directive and by the envisaged regulation on food fortification, respectively.

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2 Taxonomy, Morphology, and Ecology of Medicinal Plants

A Botanical Perspective

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CONTENTS

2.1 INTRODUCTION

The aim of this chapter was to introduce plant taxonomy and morphology and to explore why—from the perspective of the plant—biologically active compounds are produced. Thus, we shall present a view of plant morphology and biologically active compounds set within an evolutionary and ecological context. Also, we wish to dispel the traditional view that it is only the secondary metabolites of plants that can be exploited as sources of therapeutic drugs, that is, metabolites that do not contribute to primary metabolism and growth but have specialized roles, usually in plant defense or the attraction of animals for pollination or seed dispersal (Wink, 2004). A range of primary metabolites are now promising as potential therapies to fight some of the most insidious human diseases and disorders. Being plant primary metabolites, they can be readily found in great quantity.

2.2 PLANT MORPHOLOGY AND THE LOCATION OF ACTIVE COMPOUNDS

2.2.1 Basic Plant Biology and Ecology

In order to understand why, and in which parts, plants produce biologically active compounds, we must have a clear understanding of basic plant biology and how this relates to plant morphology. Plants, like fungi and animals, are eukaryotic organisms (i.e., their genetic material is bound within a cell nucleus and their cells possess membrane-bound "organelles" that are involved in producing chemical energy). However, plant cells are contained within a rigid cell wall, and multicellular tissues produce rigid organs that occupy space and acquire resources from the local environment. Plants are also the only eukaryotes to have chloroplasts (organelles specialized to perform photosynthesis). Thus, we could define plants as "a lineage of eukaryotic organisms exhibiting cell walls and chloroplasts"—a broad definition that includes everything from algae to *Zygopetalum* orchids.

The resources plants used to produce chemical energy and biomass are atmospheric carbon dioxide, light energy, and inorganic matter, including a range of minerals and water, which are used during photosynthesis in the chloroplasts. Oxygen is also used during respiration in the mitochondria to produce chemical energy. The thin gruel on which plants subsist imposes a relatively low-energy metabolism, and the rigid cell walls also contribute to a sedentary lifestyle, obliging terrestrial plants to spread via the dispersal of propagules, such as spores or seeds, or simply by clonal growth. A sedentary lifestyle also means that plants cannot flee their predators, which include various vertebrates, invertebrates, and microbes, from the largest land mammals to the tiniest viruses. Indeed, plants are the primary producers of biomass and energy in most terrestrial ecosystems, and thus have strong controlling effects on the resources available to the other organisms within ecosystems. It is for this reason that many plants, in the struggle to retain resources and energy, must defend their tissues. Producing biologically active compounds is one way of achieving this, and chemical means of defense, as we shall see later, may act against animals, microbes, and even other plants via a range of mechanisms.

Bacteria and fungi are also prodigious producers of specialized biologically active compounds, many of which, such as antibiotics, are the basis of significant modern therapies. However, the fact that plants are the major constituent of most ecosystems, they are sedentary, large enough for us to recognize and utilize, are easy and relatively safe to handle, and produce a range of biologically active compounds that can readily be extracted, means that plants offer the greatest potential as economically viable sources of active compounds.

2.2.2 General Plant Taxonomy and the Evolution of Plant Morphologies

It is important to recognize that the location of biologically active compounds within the plant depends on plant morphology—the division of the whole plant into organs such as roots, stems, and leaves—which in turn depends on plant taxonomy and the events that have occurred during plant evolution. Mosses, for instance, are primitive life forms that do not have true leaves (in which cells are differentiated into tissues specialized to perform different functions) but rather simple leaf-like structures consisting, essentially, of a sheet of general-purpose photosynthetic cells. Liverworts, the most primitive of all land plants (Sanderson et al., 2004), have no leaves and consist of a simple "thallus" or a flattened structure consisting of photosynthetic tissue sandwiched between two layers of epidermal cells, with single-celled rhizoids acting in the same capacity as roots. Indeed, land plant evolution has been characterized by a series of increasingly sophisticated changes in morphology. This can be summarized by the general sequence of plant evolution laid out in the following paragraphs. Our aim in this section is not only to highlight the development of plant morphology through time, but also to provide a general framework that will clarify the taxonomic relationships between the major groups of plants, such as mosses, ferns, conifers, and flowering plants. It is essential to understand the relationships between groups of plants because, as we shall see, many of the relatively primitive plants are now being investigated for biologically active compounds and are yielding startling results.

First, it should be acknowledged that a range of organisms such as the fungi and blue-green "algae" are traditionally viewed as plants in many botany text, but actually belong to different lineages and thus have different morphologies and physiologies. Three main kingdoms of life are currently recognized: the eukaryotes, eubacteria, and the archaea (Madigan et al., 2003). Fungi are nonphotosynthetic species that form an ancient eukaryotic group and are classified together with plants and animals though they do not belong to any of these. Blue–green algae are not eukaryotes but eubacteria (specifically, cyanobacteria) that carry out photosynthesis in essentially the same manner as plants and thus share some characteristics, particularly with plant chloroplasts. However, cyanobacteria are prokaryotes (i.e., do not have membrane-bound genetic material, nor organelles), and many aspects of their biology are thus quite distinct from those of plants. Lichens are a symbiotic mixture of fungi with either algae (mainly green algae) or cyanobacteria and thus have a rather odd taxonomic status.

We have already defined plants as "a lineage of eukaryotic organisms exhibiting cell walls and chloroplasts." Indeed, plants form a single lineage of eukaryotes that include, at their base, the marine and freshwater algae. The earliest known fossil alga, known as *Grypania*, had a morphology equivalent to that of a modern green alga (Chlorophyceae: Charales), and although other algal groups such as the red algae and the brown algae have persisted to the present day, it is only the green algae from which land plants evolved (Willis and McElwain, 2002).

The first plants to live on land were simple green algae that probably lived along humid riverbanks on thin soils formed by microbial activity. Around 477 million years ago (m.y.a.), this situation allowed the evolution of the first plants that were truly adapted to live on land: the liverworts (Marchantiopsida). These usually had (and still have) a flattened thallus with rhizoids on the undersurface and simple pores on the upper surface. This simple morphology reflects life at the interface between soil and air and is efficient for maximizing surface area-to-volume ratios for efficient gas exchange, water uptake, and for intercepting sunlight.

The first stem-like structures were photosynthetic but their main function was to hold spore-bearing structures aloft for spore dispersal and can be found in liverworts and their close relatives the hornworts (Anthocerotopsida). Structural support depended on a central core of hydrostatically pressurized cells encased in a rind of photosynthetic tissue. Simple stem-like structures are also a feature of the mosses (Bryopsida), which evolved around 45 million years after the liverworts. Mosses are able to capture rainwater and dissolved mineral nutrients, soaking them up among their "leaves," and this accumulation of nutrients accelerated soil development and is thought to have fueled an explosive burst of plant evolution around 432 m.y.a. (Pierce et al., 2005).

This explosion resulted in the evolution of specialized lignified (strengthened) mechanical tissues and ultimately vascular bundles (in which lignified water-conducting tissues are associated with mechanical tissues) in the first true vascular plants (Tracheophytes), known as the Rhyniopsida. These were capable of the internal transport of water, nutrients, and the products of photosynthesis over much larger distances, and plants started to become large. None of these early vascular plants has survived until the present day and are only relevant to our discussion in terms of understanding how true stems came about and are structured to perform their role as supporting organs.

Differentiation into multicellular tissues allowed the production of long roots that anchored the plant more effectively and "foraged" for resources such as soil water and inorganic nutrients and, eventually, also acted as storage organs for carbohydrate reserves. True leaves, like true roots, are differentiated into specialized tissues, and probably evolved sometime between 390 and 354 m.y.a. (Willis and McElwain, 2002). Club mosses (Lycopodiaceae), *Selaginella* and *Isoetes*, are surviving examples of an ancient group of vascular plants that have microphyll leaves (i.e., leaves that have only a single strand of vascular tissue and are attached directly to the stem). Megaphyll leaves have many vascular strands and may be much broader and are characteristic of horsetails (Sphenopsids), ferns (Filicophyta), and seed plants (Spermatophyta). Around 330 m.y.a., ferns and early seed plants developed particularly broad, laminar leaves because the widespread vegetation had removed substantial quantities of CO_2 from the global atmosphere. This meant that plants needed more stomata (adjustable pores) over a greater surface area than was available on their photosynthetic stems, in order to assimilate enough CO_2 to perform photosynthesis. Greater numbers of stomata also allowed greater evaporation and thus more efficient cooling, meaning that for the first time large photosynthetic structures exposed to direct sunlight did not dangerously overheat (Beerling et al., 2001).

Woodiness (secondary growth) evolved at around the same time because the evolution of large leaves resulted in intense competition for light, and height became a distinct advantage in many ecosystems. All plants possess the structural compound lignin, used to reinforce cell walls and thus tissues, and in woody plants, lignin is used to produce particularly sturdy vascular and structural tissues. However, in order to grow in height the stem must be stable, meaning that it must also have a mechanism for growing in girth, despite its solidity. The term secondary growth refers to this lateral expansion of woody stems, which is based on the laying down of fresh cells over the flanks of the stem by a cylinder of differentiating cells called the cambium, or lateral meristem, which lies just beneath the bark. This results in the formation of wood, which is a distinguishing characteristic of the early Eutracheophyta.

Also around 380 m.y.a., all sexually reproducing plants had at least one phase of the life cycle that depended on sperm cells swimming in the water films covering plants and soil, in order to fertilize the egg cells of neighbors. Thus, vegetation was restricted to humid environments, despite the fact that the plants themselves included large tree-like growth forms that formed extensive forests. The last link between plant life and humid habitats thus took place with the evolution of pollen (essentially sperm that is coated to resist drying, to travel on the wind) and, contemporaneously, seeds. Seeds are composed of an embryo protected by a tough seed coat and, typically, a store of reserves to fuel embryo growth during germination.

As forests invaded drier habitats, and thus covered most of the land surface, all of the basic ways of growing had evolved, leaving scope for only relatively minor variation around the theme of the general land plant body-plan. However, there was still room for the improvement of gymnosperm reproductive systems, and the evolution of flowers and fleshy fruit, probably around 217 m.y.a. (Smith et al., 2010), allowed early flowering trees to survive in forests already dominated by large tree life forms, where they languished for around 75 million years before becoming common.

The innovative and defining feature of flowering plants was not actually the flowers, but the fact that the egg was completely enclosed and protected within an ovary (it is from this that we get the name Angiosperm; *angio* meaning cup, representing the ovary, and *sperm* meaning seed). It is the ovary that develops, alongside the seeds, to form the surrounding fruit, which may be fleshy and attractive to animals that disperse the seeds and thus important for animal nutrition. Petals are simply modified leaves that advertise the presence of pollen: the first simple flowers offered no nectar reward for pollinators; it was the pollen itself that visiting beetles ate, some of which was accidentally transported from flower to flower in a kind of pollination known as *mess-and-spoil* due to the general defacement sustained by the plants. Another theory of flower evolution suggests that the volatile secondary metabolites produced to protect delicate regenerative organs against disease organisms were similar to many insect hormones, resulting in the use of gymnosperm reproductive organs as sites for insect mating, with pollen of lesser importance as an attractant (Harrewijn et al., 1995)—although the bright yellow color of pollen is undoubtedly attractive to many modern insect pollinators. Nectar evolved later as part of symbioses with particular insects such as bees and butterflies.

We have, so far, described the general sequence of events leading to the evolution of the different parts of plants: thalli and rhizoids, stems, roots, leaves, wood, pollen, seeds, and ultimately fruits and flowers. We have also seen that the evolution of these morphological features defines the taxonomy of the major plant groups. Thus, it should also be evident that not all plants will possess all of the organs that we may typically associate with plants and that the morphologically primitive taxa that still exist today offer a more limited range of growth forms and organs that can be exploited for medicinal purposes.

However, most plants possess photosynthetic tissues, and it is usually these that are exposed to attack and are thus relatively rich in secondary metabolites (Harborne, 1997). Among the vascular plants, buds and storage organs that must persist in hostile surroundings, such as tubers and rhizomes (underground stems), may also be particularly rich sources of secondary metabolites, as are young, expanding organs which do not yet enjoy the protection of highly lignified (and thus hardened) cell walls. Flowers, which are usually relatively ephemeral, are typically poor sources of secondary metabolites involved in defense, as they require relatively little protection (Harborne, 1997), although they may be unique sources of volatile fragrance compounds. Fruits are also poor in defense compounds, being rich in secondary metabolites such as pigments (particularly anthocyanins and carotenoids) in order to attract animal dispersal vectors, but the seeds themselves may be rich in tannins to deter ingestion (e.g., grape seeds). The pigments are antioxidants and are thus an important part of the diet.

2.3 DISTRIBUTION OF MEDICINAL PLANTS AND ACTIVE COMPOUNDS AMONG THE PLANT KINGDOM

Here we shall argue that biologically active compounds are a general feature of the plant kingdom because they originally evolved to aid the survival of the earliest plants. Indeed, we shall concentrate on the compounds produced by the more "primitive" plants and conclude with a discussion of the general trends in occurrence and evolution of secondary metabolites.

That plant defense compounds are widespread is more than simply an intriguing phenomenon; it may actually have played a key role in the development (or more correctly, hindrance to development) of human civilizations. Most leaves and tubers of wild plant species are potentially toxic to humans, and only through domestication have concentrations of toxic compounds been reduced in cultivated varieties (Harborne, 1997). Presumably, the first people to domesticate toxin-laden plants such as the potato were faced with desperate food shortages. Artificial selection (breeding) has resulted in weak plant defense in domesticated crops and the need for human intervention to ensure the survival of many domesticated plants (Wink, 2004).

Three principal groups of secondary metabolites are involved in plant defense: nitrogen-containing substances, terpenes, and phenolics (Wink, 2004). The first group includes alkaloids, amines, cyanogenic glycosides, glucosinolates (mustard oils), peptides, and specific anti-feedant proteins such as protease inhibitors and amylase inhibitors. Approximately 14,000 have been described to date, the vast majority of which are alkaloids (Wink, 2004). Alkaloids possess a heterocyclic nitrogen that can act as a base and interfere with neuronal signal transduction pathways, having toxic effects on animal nervous systems. Terpenes are aromatic compounds, the structure of which may include multiple aromatic ring formations (e.g., diterpenes, triterpenes, and sesquiterpenes), and in certain forms can interfere with the properties of cell membranes, making them leaky and thereby nonfunctional. Iridoids are a particularly widespread group of monoterpenes. Phenolic compounds, including flavonoids, bisbibenzyls, and tannins, have a range of sinister properties, such as forming cross-linkages between DNA bases and proteins, and many are thus mutagenic and carcinogenic. Indeed, if there is a common concept linking the disparate types of biologically active compounds, it is the disruption of the structure, and thus function, of the cellular components (membranes, proteins, genetic material) that are crucial to the primary metabolism of organisms that are antagonistic to the plant—it is for this reason that many have general antibiological effects against a range of animals, micro-organisms, and other plants.

Incidentally, in order to avoid poisoning themselves with their own secondary metabolites, plants must usually separate the precursors of secondary metabolites in different tissues. When tissues are disrupted during herbivory/disease, the precursors are mixed together or mixed with enzymes that cleave particular parts of the precursor molecules to produce the toxic form (e.g., iridoid glucosides become exposed to the enzyme β-glucosidase, which removes the glucose group to produce a toxic iridoid aglucone; Rank et al., 2004).

Most plants share these basic classes of secondary metabolites, even the most ancient of all: the green algae.

2.3.1 Green Algae

Green algae, which we have seen are the progenitors of land plants, may contain a range of biologically active compounds, including diterpenes that are toxic to bacteria (Ahmad et al., 1994), and thus probably defend the plant against disease. Paul and Fenical (1986) investigated 40 species of multicellular green algae and found that they produced a range of terpenes of unusual and distinctive types that are toxic to micro-organisms, sea urchin larvae, molluscs, and fishes. These compounds were produced in greater concentrations when herbivory was more intense, and in particular in the softer and more vulnerable growing points of organs, although there was no difference in the concentration between different organs. They concluded that biologically active compounds are produced in order to defend tissues against herbivory. Paul et al. (1987) then confirmed that sesquiterpenes in *Caulerpa ashmeadii* deter feeding by herbivorous fishes. Similarly, the marine green alga *Bryopsis maxima* produces (Z)-8-heptadecene (which has a range of suppressive effects on herbivorous marine invertebrates) only when wounded (Akakabe et al., 2007). In temperate waters, the green alga *Ulvaria obscura* can sometimes form “blooms” that choke subtidal marine communities, mainly because of its toxicity and thus lack of grazing by invertebrates. This alga produces dopamine, a common neurotransmitter in animals, that interferes with animal nervous systems and thus acts as a feeding deterrent (Van Alstyne et al., 2006). Leflaive and Ten-Hage (2007) review evidence that green algae also produce alkaloids that are used to deter herbivores and fatty acids and as-yet unidentified compounds to inhibit competing algae and aquatic flowering plants (note that the chemical suppression of competitors is known as "allelopathy" and is also known for flowering plants). Indeed, blooms of the green alga *Botryococcus braunii* follow the excretion of free fatty acids that inhibit growth of both zooplankton and phytoplankton (i.e., suppressing both herbivores and competitors; Chiang et al., 2004). Algae produce greater concentrations of allelopathic compounds in conditions of stress, induced by suboptimal nutrient availability, pH, or temperature, and also in response to the presence of waste compounds produced by competing species—this can alter the structure of the algal community (Leflaive and Ten-Hage, 2007, and references therein).

Thus, green algae, which essentially live in a microbial soup and are also surrounded by animal enemies and plant competitors, use secondary metabolites to defend themselves against disease and herbivory and to increase their chances of competitive success. Land plants have inherited these survival tools.

2.3.2 Liverworts

The most primitive land plants can produce sophisticated chemical defenses that can also potentially be exploited for medicinal purposes. The liverwort *Dumortiera angust*, like many other liverworts, produces marchantin C (a phenolic bisbibenzyl), which is an antioxidant and antimicrobial and has been shown to cause apoptosis (programmed cell death) by regulating gene expression in various types of human cancer cell (Shi et al., 2008). Marchantin C is also able to arrest the growth of human cervical cancer cells by disrupting the form and function of microtubules that are crucial to cell division (Shi et al., 2009). A similar ability to induce apoptosis in human breast cancer cells has also been noted for marchantin A, isolated from *Marchantia emarginata* (Huang et al., 2010). A range of other liverwort species contain other bisbibenzyls with general antimicrobial activity (e.g., *Marchantia polymorpha* (Niu et al., 2006; Mewari and Kumar, 2008); *Schistochila glaucescens* (Scher et al., 2002)) and terpenoids (e.g., *Conocephalum conicum* and *Dumortiera hirsuta* (Lu et al., 2006)). Indeed, the liverworts *Asterella blumeana*, *Lunularia cruciata*, and *Scapania undulata* contain sesquiterpenes and flavonoids that function as antioxidants and exhibit antimicrobial activity (Basile et al., 1998; Ielpo et al., 1998; Neves et al., 1998; Adio et al., 2004).

2.3.3 Hornworts

Hornworts such as *Anthoceros agrestis*, *Anthoceros punctatus*, and *Folioceros fuciformis* also contain rosmarinic acid (an antibacterial, antiviral, and antioxidant phenolic compound; Zelić et al., 2005), rosmarinic acid 3′-*O*-β-D-glucoside (Vogelsang et al., 2006) and alkaloids (Trennhäuser, 1992; Trennhäuser et al., 1994). *Anthoceros caucasicus* is known to contain sesquiterpenes (Mekem Sonwa and König, 2003).

2.3.4 Mosses

Veljić et al. (2009) tested the extracts of eight moss species, representing seven families, and found antibacterial and antifungal activities in all, although it is not known which particular secondary metabolites were involved. Some mosses are known to contain terpenes (Saritas et al., 2001).

2.3.5 Clubmosses

A range of alkaloids, terpenes, and phenolic compounds are produced by clubmosses (Liu et al., 2004, and references therein; Shi et al., 2005; Srivastava et al., 2008). These have traditionally been employed as painkillers and for burn treatments (Srivastava et al., 2008), and some also promise treatment for Alzheimer's disease (Tang, 1996). Indeed, clubmoss extracts have demonstrated anti-inflammatory properties and antibacterial and antifungal effects in the laboratory (Orhan et al., 2007a, 2007b). The traditional use of *Lycopodium serratum* for wound healing has recently received support from the finding that extracts do improve skin integrity during the healing process, at least when used on rats (Manjunatha et al., 2007).

2.3.6 Ferns

Ferns contain a range of compounds including flavonoids, terpenes, phloroglucinols, and xanthones (Soeder, 1985). Terpenes are common, being found in a range of fern life forms from aquatic species (*Azolla nilotica*, *Azolla japonica*; Arai et al., 1998; Nakane et al., 2003), herbs (*Thelypteris hispidula*, *Woodwardia virginica*; Hanu et al., 2003; Socolsky et al., 2005) to tree ferns (*Lophosoria quadripinnata*; Tanaka et al., 1992). Bracken (*Pteridium aquilinum*) produces flavonoids, sesquiterpenes, echydones, cyanogenic glycosides, tannins, and phenolic acids (Cooper-Driver, 2008). Indeed, it is the ptaquiloside (a sesquiterpene) of bracken that can cross-link DNA and proteins, resulting in bladder cancer in humans and livestock (Wink, 2004).

2.3.7 Seed Plants and General Trends in Secondary Metabolite Occurrence and Evolution

Some, but by no means all, secondary metabolites appear to have become increasingly sophisticated throughout evolutionary time, and may be particularly advanced and variable among the seed plants. However, our knowledge is so incomplete that it is dubious whether or not tracing the evolution of certain classes of secondary metabolite provides an accurate picture. For instance, alkaloids are traditionally seen as advanced secondary metabolites produced by flowering plants (Harborne, 1993), but we have already seen that green algae, hornworts, and clubmosses may produce alkaloids (Trennhäuser, 1992; Trennhäuser et al., 1994; Liu et al., 2004; Leflaive and Ten-Hage, 2007). Similarly, the fact that rosmarinic acid is produced by a number of hornworts and ferns (Häusler et al., 1992; Vogelsang et al., 2006) is at odds with the traditional view that this compound is exclusive to flowering plants of the families

Lamiaceae and Boraginaceae. Our view of the distribution of secondary metabolites among the plant kingdom may be heavily skewed in favor of the relatively widespread flowering plants that have been more commonly studied.

Even within the flowering plants, it is currently extremely difficult to comment on the trends in the evolution of specific secondary metabolites within or between families. For example, cucurbitacins are a group of active compounds that are usually isolated from species of one particular family, in this case the Cucurbitaceae (cucumbers, melons, squashes, etc.). This has erroneously given the impression that cucurbitacins are unique to this family. There is a growing recent literature centered around the description of new cucurbitacins from novel source species and the fact that these compounds may have antibacterial and anticancer activities (e.g., Sun et al., 2010). Most of these new sources are indeed Cucurbitaceae, but a wider search is now revealing that cucurbitacins also occur in members of the Scrophulariaceae (e.g., Smit et al., 2000; Wang et al., 2004; Zou et al., 2004; Allen et al., 2006; Kim et al., 2006; Bhandari et al., 2007; Kaya and Melzig, 2008), Rosaceae (Sarker et al., 1999; Munoz et al., 2000, 2002; Maloney et al., 2008), Sterculiaceae (Chen et al., 2006), Rubiaceae (Litaudon et al., 2003) and Elaeocarpaceae (Ito et al., 2002; Rodriguez et al., 2003), and thus appear to have evolved either a number of times in different groups or once in a common ancestor without being expressed in many species.

These studies have one thing in common: they have been conducted recently in (mainly) tropical floras for which the occurrence of secondary metabolites is little known, and where research into secondary metabolites and medicinal plants is a growing field for local science. In other words, attempts to describe the general occurrence of particular secondary metabolites among the plant kingdom may be premature because studies have historically been biased toward temperate, particularly European, flowering plants.

We do have a clear view of the evolution and occurrence of certain secondary metabolites. Mustard oils, for instance, are known to be restricted to 14 families that are closely allied to Brassicaceae (together these families form the order Capparales), based on molecular and morphological data (Rodman et al., 1996). As far as is known, only once has convergent evolution resulted in the appearance of mustard oils in a family not closely related to the Capparales—in the tree species *Drypetes natalensis* (Putranjivaceae; Johnson et al., 2009). Jensen et al. (2007) also advocate an approach similar to that of Rodman et al. (1996): that is, that combined use of DNA sequence data, plant morphology, and secondary metabolites can form a powerful tool for precisely discerning the relationships between closely related taxa. However, while these techniques allow investigation of the fine details of secondary metabolite evolution in particular cases, it is the broader view that we currently lack.

Comprehensive investigations of an entire class of secondary metabolites, such as that of Rodman et al. (1996), are rare. Even within the most common flowering plant families, our ignorance of plant secondary metabolites is profound. Only 0.5% (136 out of ~25,000 species) of one of the largest families, the Asteraceae, are included in the most recent edition of the authoritative *Codex Vegetabilis* (Proserpio, 1997; originally published as Steinmetz 1947). Within this subgroup, a range of compounds is evident: 67 species are valued as a source of essential oils, 9 species produce alkaloids, 8 glucosides, 28 tannins, 15 terpenes, 29 flavonoids, and a small number of species contain combinations of these compounds. Proserpio (1997) includes 109 Fabaceae (legumes), 19 of which contain alkaloids, 32 tannins, 27 essential oils, and a smaller number contain flavones, resins, or glucosides. In contrast, of the 73 species of Lamiaceae recorded as medicinal species, most (84%) are employed for their essential oils. Thus, different families tend to contain different numbers of species valued for particular classes of compound, but nonetheless a great range of compounds is present within each family. Furthermore, the above examples are based on extremely small sample sizes, insufficient to provide a comprehensive overview of the trends in the evolution of secondary metabolites within these families, and yet they are among the largest and, for medicinal plants, most widely utilized of flowering plant families.

In conclusion, these examples illustrate that while we may be able to discern the details of secondary metabolite evolution between particular taxa, attempts to broadly trace the evolution of active compounds are, in our opinion, currently inadequate. Ongoing investigation, particularly of ancient plant lineages and tropical floras, is revealing that supposedly advanced secondary metabolites, or metabolites that were once thought to be highly restricted to particular flowering plant lineages, may actually be found in a much greater range of taxa. For example, "the Anthocerotae [hornworts], like the large group of the Musci (mosses) . . . are considered to be poor in terpenoid metabolites. However, after the presence of many terpenoid compounds was reported in Musci, it appeared worthwhile to have a closer look at the volatile constituents of the Anthocerotae as well" (Mekem Sonwa and König, 2003), which, indeed, they went on to find. We suspect that there are many such instances in which an apparent lack of particular secondary metabolites in certain plant groups is simply an artifact of our lack of enquiry, rather than any deficiency on the part of the plant. This provides a message of hope for those wishing to exploit plant secondary metabolites for therapeutic purposes: the implication is that medicinal botany currently relies on a minuscule proportion of the biologically active compounds that exist in nature.

2.4 PLANT DEFENSE, THE COSTS OF SECONDARY METABOLITES, AND ECOLOGICAL STRATEGIES

2.4.1 Trade-Off between Direct and Indirect Defense

Most secondary metabolites are involved in the defense against generalist herbivores and opportunistic disease organisms. For instance, *Capsicum annuum*, the most common of the five domesticated species of peppers (Ince et al., 2010), includes a range of secondary metabolites such as carotenoid pigments (which attract animal seed dispersal vectors) and polyphenols (quercetin, luteolin, and capsaicinoids—it is the capsaicinoids that vary most between varieties and give hot chillies their heat; Saga and Sato, 2003). These polyphenols are antioxidants, which incidentally have promising antimutagenic properties (Nazzaro et al., 2009), and in nature have a range of toxic effects on micro-organisms (Cichewicz and Thorpe, 1996) and insects (Cardoza and Tumlinson, 2006). However, there exist cases in which animals have evolved resistance to defense compounds and actually prefer to eat plants containing these compounds because there are fewer herbivores competing for that particular food source. Thus, humans are not the only animals that seek chillies—the American pepper weevil (*Anthonomus eugenii*) can smell the plume of volatile compounds given off by the plant and use this to navigate to its food source (Addesso and McAuslane, 2009). (For the record, birds are the main seed dispersal vector of chillies in nature, are attracted to the brightly colored fruits, and do not have the same pain/heat receptors as mammals and thus are not deterred by their pungency—this is important for successful seed dispersal as mammals grind seeds whereas birds swallow food whole and let the seeds pass through the gut.)

Despite the apparently disadvantageous effect of advertising to insects, the main function of this system of volatile emission is actually to attract insects, but typically as part of an inducible defense system. Herbivore damage, particularly by generalist invertebrates such as spider mites, stimulates the emission of volatile terpenes, which attract specific carnivorous insects (van den Boom et al. 2004). Thus in addition to direct defense (i.e., simply being full of noxious chemicals) peppers also use a form of indirect defense mediated by a third party; the predator. Plants thus face a trade-off between investment of resources in direct and indirect defenses. Despite exhibiting moderately strong direct defense, peppers actually invest more in indirect defense, which has been demonstrated to have a greater effect on herbivore behavior (Van den Boom et al., 2004). In contrast, a number of plants such as *Ginkgo biloba* invest extremely heavily in direct defense, with indirect defense having a relatively weak effect on herbivores.

2.4.2 Trade-Off between Primary and Secondary Metabolism

The concept of trade-offs is essential to plant ecology because the resources available to plants are limited and plant survival depends on optimizing resource use. Thus, another important trade-off exists with regard to resource investment in either primary metabolism (favoring growth) or secondary metabolism (favoring resistance). Indeed, Harborne (1997) states that plants can be considered as belonging to three main groups: (1) perennial "growth-dominated" plants with rapid growth, poor chemical defense but with a highly inducible defense system, (2) perennial plants with slow growth rates that are inherently well defended (often including spines and physical defenses) but with less responsive facultative defenses, and (3) annual plants for which defense is not as important. These three extremes in survival strategy reflect the three main directions in which all organisms may be adapted to survive. Indeed, Grime and Pierce (2011) have recently shown that organisms from every branch of the tree of life face a universal three-way trade-off between C-selection (competition selects for a strategy that invests resources in acquiring more resources), S-selection (stress selects for the investment of resources in the maintenance of individuals), and R-selection (disturbance selects for survival based on regeneration and a rapid lifecycle). Thus, the extent to which a plant species invests in facultative (induced) or obligate defenses reflects the overall survival strategy.

2.5 PRIMARY METABOLITES WITH THERAPEUTIC POTENTIAL

Ecological and evolutionary considerations are of much lesser importance to the occurrence of primary metabolites within the plant kingdom for the simple reason that primary metabolites are so essential for the functioning of all plants that they are ubiquitous and their functions in plant physiology are also well understood. Primary metabolites include carbohydrates, fats, proteins, vitamins, and the pigments involved in photosynthesis. These are, of course, of greatest importance to humans nutritionally, but some blur the line between nutrition and medicine and others appear to be useful as medicines in their own right, especially when artificially modified from their naturally occurring forms. The following is not an exhaustive list, as this is a recent and rapidly expanding field of research; we merely aim to highlight the concept of primary metabolites as sources of biologically active compounds with therapeutic potential. Here we showcase some particularly noteworthy recent examples that could provide cheap and abundant sources of anticancer, antiviral, and antibacterial drugs and even help against neurodegenerative disorders.

2.5.1 Pectin

Pectin is actually not a single substance but a complex macromolecule composed of a number of different types of "pectic polysaccharides" that together form a tangle of molecular strands. These are essential for the integrity and elasticity of the plant cell wall and perform the role of intercellular "glue," bonding plant cells together to form tissues. While pectin is a component of all plants, fruits may have extremely high pectin contents, and citrus peel and the solid wastes from apple juice production are the main industrial sources of pectin. However, it can also be readily isolated from Sycamore (*Acer pseudoplatanus*) (Marfà et al., 1991) or red wine (Pellerin et al., 1996).

Pectin is an important form of dietary fiber, and as an emollient, it can ease the digestive tract and is used for anti-diarrhea treatment in the form of bismuth subsalicylate. However, pectin has recently revealed a new face. It has been discovered that while citrus pectin—a naturally occurring form found in the diet—has no anti-tumor properties, when a little heat is applied or the pH altered, the strands of pectin re-arrange themselves to produce a different conformation. This modified citrus pectin interferes with cell-to-cell interactions mediated by cell surface molecules that bind to carbohydrates. This confers anticancer activity against rat and human prostate carcinomas (Pienta et al., 1995; Jackson et al., 2007), human breast carcinoma cells, and human colon carcinoma cells (Nangia-Makker et al., 2002) *in vitro* or when human cells are cultured in animals, but has yet to be tested *in vivo* in humans.

2.5.2 Lignin

Lignin is another highly complex macromolecular component of cell walls, and we have seen that it is essential for the rigidity of stems and thus the ability of plants to grow in height. It is thus particularly common in woody plants, particularly in stems and other solid structures such as pine cones. Indeed, the main source of lignin for medicinal purposes is the wood of Japanese Cedar (*Cryptomeria japonica*), Japanese Beech (*Fagus crenata*), Rice husks (*Oryza sativa*), and Bamboo (*Phyllostachys bambusoides*) (Akao et al., 2004). Certain lignins (to be precise, particular configurations of the highly complex lignin "network") are known to have an activity against human immunodeficiency virus and cancerous cells (Akao et al., 2004, and references therein), but the tough nature of lignin and the mechanical processes that are used to extract it result in an inconsistent end product that is difficult to mass-produce.

However, novel chemical methods of extracting lignins are being developed, using acids and phenols to extract lignin in the form of "lignophenols." These lignin derivatives are known to protect human neurons, *in vitro*, against reactive oxygen species and, although this has yet to be tested *in vivo*, encouraging early results suggest that lignin could potentially find a therapeutic role in delaying the progress of neurodegenerative disorders such as Parkinson's, Alzheimer's, and Huntington's diseases (Akao et al., 2004).

2.5.3 Chlorophylls

Chlorophylls are of course pigments that are essential for the process of capturing light energy during photosynthesis. Being antioxidants, they are also a useful component of the human diet, but recently it has been found that naturally occurring chlorophylls and a chlorophyll derivative, sodium copper chlorophyllin (SCC), also confer a degree of protection against a range of cancer-causing agents *in vitro* (reviewed by Ferruzzi and Blakeslee 2007). Natural chlorophylls extracted from Spinach (*Spinacia oleracea*) and SCC both have proven ability to reduce mutagenic effects *in vivo* in Rainbow Trout livers (Harttig and Bailey, 1998), and a number of studies have shown that SCC is effective *in vivo* against skin cancer in mice (Ferruzzi and Blakeslee, 2007, and references therein). This appears to be a result not only of the antioxidant properties of chlorophyll but also of the ability to induce apoptosis, demonstrated for SCC acting on human hepatocellular carcinoma cells (Chan et al., 2006) and human colon cancer cells (Diaz et al., 2003). However, a range of positive and negative effects of natural chlorophylls and SCC has been observed for colon cancer in rats, and the particular combinations of chlorophyll form and dosage that are beneficial remain an unknown factor that currently hinders chlorophyll use as a drug.

We now know that the traditional view that the human gut does not absorb chlorophyll derivatives is flawed, as blood plasma of patients taking SCC supplements contains SCC-derived compounds, and gut cells have now been shown to absorb chlorophyll derivatives (Ferruzzi and Blakeslee, 2007). Further encouragement regarding the therapeutic potential of chlorophyll against human liver cancer *in vivo* comes from a randomized, double-blind, placebo-controlled trial in Qidong in China, where inhabitants are exposed to carcinogenic aflatotoxin B1. For four months, participants in the trial consumed supplements of 100 mg SCC three times daily, which resulted in a 55% reduction in aflatoxin-N7-guanine excreted in the urine. Ferruzzi and Blakeslee (2007) suggest that while this is heartening it might be simpler, and just as effective, to eat plenty of green vegetables.

2.5.4 Should Primary Metabolites and their Derivatives be Regulated?

One perceived advantage of primary metabolites as medicines is that these compounds are an unavoidable part of every diet and have therefore been eaten by humans for the entire history of the species—they have an exemplary safety record and are usually exempt from regulation. However, artificially modified forms and derivatives, such as lignophenols, have never been a part of the human diet. Conversely, some derivatives of plant primary metabolites that have always been a part of human diets, such as alcohol, can undoubtedly incur health risks and are currently regulated. It may be wise to ask ourselves what the possible human health implications of primary metabolite derivatives are, and whether compounds that seem familiar, and for which it is intuitive to assume are benign, are truly trustworthy.

2.6 CONCLUSIONS

Throughout the plant kingdom, from the lowliest alga to the loftiest tree, there exist a vast diversity of biologically active compounds that originally evolved to defend algae, and then land plants, against herbivores, disease, or other competing plants. However, it has only recently become appreciated that the primary metabolites that all plants must produce for their basic functioning and growth can also provide a range of readily available biologically active compounds that could potentially be developed into relatively economical therapies even for some of the most pernicious human diseases.

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3 Ethnopharmacological Approaches Used to Identify Medicinal Plants

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CONTENTS

3.1 INTRODUCTION

Plant-derived drugs have played an important role in the health and wellness benefits. Traditional practice may include psychological, spiritual, and cultural elements, as well as medical elements. Despite the increasing use of herbal medicines, there is still a significant lack of research data in this field. A survey of the literature revealed that the plant has been recommended for use in various traditional systems of medicine for the treatment of diseases. It is becoming increasingly evident that herbs and botanicals are beneficial to the human health. Of particular interests are analgesic and inflammatory properties. There are a number of published articles medicinal plants selected by WHO (2007), or plant-derived activities may function as an analgesic and anti-inflammatory agent. A large number of researches have determined the degree of clinical support for the traditional use or folklore medicines. Such evidence is important to determine whether there is a sufficient scientific data for their use to cure the diseases. However, we were still unclear which plant extracts worked like a clinical analgesic and anti-inflammatory agent.

The purpose of this review is to provide a short ethnopharmacological overview together with behavioral antinociceptive (analgesic) assessment in pharmacological applications and to highlight various assessment methods of analgesic properties of medicinal plants. Using this assessment, we hoped to determine the degree of support for each specific extract of medicinal plants.

3.2 INFORMATION OF MEDICINAL PLANTS OBTAINED

The following types of information were obtained from *WHO Monographs on Selected Medicinal Plants* (World Health Organization, 2007): definition, synonyms, selected vernacular names, geographical distribution, description, plant material of interest, general identity tests, purity tests, chemical assays, major chemical constituents, medicinal uses, pharmacology, adverse reactions, and contraindications. The next step was to pick up the plants that possess pharmacological