3.12 Ethnopharmacology and Drug Discovery

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### 3.12.1 Introduction

Artemisinin, triptolide, celastrol, capsaicin, and curcumin are poster children for the power and promise of turning traditional medicines into modern drugs. However, their stories highlight the ongoing interdisciplinary research efforts that continue to be necessary to realize the pharmaceutical potential of traditional therapeutics.¹

As highlighted by Corson and Crews,¹ drug development in its modern understanding focuses on pure chemical entities, and local and traditional knowledge remains an essential starting point for such research and development (R&D).

There can be no doubt that observational knowledge about the effect of a plant, an animal, or a microorganism on other organisms offers ideal opportunities to limit the huge diversity of possible leads to more promising ones (knowledge-based drug discovery). Such observational knowledge is exemplified by the discovery of penicillin (Alexander Fleming, 1928) and by the discovery of desmoteplase, a protein recently isolated from vampire bats (which need it to prevent their prey’s blood from coagulating), which was developed to treat the effects of strokes.²

The ethnopharmacological approach is unique in natural product research in that it requires input from the social and cultural sciences. It is essential to distinguish two parts of these development activities: the field-based study of local resources, or the documentation of practitioners’ healing practices, and the bioscientific study of this knowledge and of the products used.

In many regions of the world, knowledge was or still is mostly passed on orally from one generation of healers to the next. This knowledge has been the focus of researchers who have been called ethnobotanists or ethnopharmacologists. On the contrary, there are written records from practitioners from cultures such as the
Chinese, Arabic, Asian Indian, Mexican Indian (Aztec), and, of course, European traditions who wrote down their knowledge.

In 1896, the term ‘ethnobotany’ was coined by the American botanist William Harshberger describing the study of plant use by humans. The term is generally based on a detailed observation and analysis of the use of plants used in a society and of all beliefs and cultural practices associated with such use. Ethnobotany and ethnopharmacology investigate the relationship between humans and plants in all its complexity. Ethnobotanists live with members of a community, share their everyday lives, and, of course, respect the cultures of the host. Ethnobotanists have a responsibility not only to the scientific community but, equally important, also to indigenous cultures. A complex set of methods are used that are derived from the social and cultural sciences (including taking detailed field notes, quantitatively assessing reported uses, cognitively and symbolically analyzing plant usage) and the natural sciences (collecting plant samples – voucher specimens – that allow precise determination of the plant species). Ethnobotanical studies have many theoretical and applied interests; in fact, only a very few are in any way directly linked with projects in the area of drug discovery.3–7

Ethnopharmacology as a specifically designated field of research has had a relatively short history. The term ‘ethnopharmacology’ was first used in 1967 as the title of a book on hallucinogens: Ethnopharmacological Search for Psychoactive Drugs.8 However, it would be meaningless to limit this discussion to the period after 1967. Medicinal plants are an important element of indigenous medical systems in many parts of the world, and these resources are usually regarded as part of the traditional knowledge of a culture; thus, any study that focuses on the documentation and systematic study of local and traditional uses of a plant or a group of plants can be considered to have ethnopharmacological relevance. Explorers, missionaries, merchants, but also knowledgeable experts in the respective healing, tradition, describe the uses of such medicinal plants; all this is the basis for ethnopharmacology-based drug development. Although such knowledge has been widely used for centuries as a starting point for drug development, once an initial lead is found, many researchers no longer consider this knowledge to be relevant. Unfortunately, the oral tradition of medical knowledge is often simply ignored as in a classic review of the drug development process, W. Sneader’s Drug Discovery: A History.9

Clearly, natural products remain one of the most important sources (or maybe even the most important one) of new drug leads. As Chin et al.10 have pointed out, more than half of all new chemical entities launched in the market are natural products or their derivatives or mimetics. This review is thus not about drug discovery from natural sources, a topic that has received considerable attention in recent years,10–17 but specifically on the link between local/traditional knowledge (or what could also be called botanical therapeutics18) and drug development.19–22

3.12.2 ‘Old’ Drugs – New Medicines

Drug development and discovery as we know it today is an outcome of the Enlightenment in Europe and the rapid expansion of pharmaceutical industries, which started in the second half of the nineteenth century. Up to this point, medical treatment strictly relied on crude materials obtained from nature and their extracts that were processed and formulated into medicines.23 The nineteenth century was when researchers began to characterize pure chemical entities in medically used or toxic plants and other organisms.

3.12.2.1 The Late Eighteenth and the Nineteenth Century

The study of the botanical origin of the arrow poison curare, its physiological (as well as toxic) effects, and the compound responsible for these provides a fascinating example of an early ethnopharmacological approach. Curare was used by ‘certain wild tribes in South America for poisoning their arrows’.24 Many early explorers documented this usage. Particularly well known are the detailed descriptions of the process used by Alexander von Humboldt in 1800 to prepare poisoned arrows in Esmeralda, Venezuela, on the Orinoco River. There, von Humboldt met inhabitants who were celebrating their return from an expedition to obtain the raw material for making the poison. Von Humboldt then describes the ‘chemical laboratory’ used:
He [an old Indian] was the chemist of the community. With him we saw large boilers (Siedekessel) made out of clay, to be used for boiling the plant sap; plainer containers, which speed up the evaporation process because of their large surface; banana leaves, rolled to form a cone-shaped bag [and] used to filter the liquid which may contain varying amounts of fibres. This hut transformed into a laboratory was very tidy and clean (von Humboldt,24 p 88).

As early as 1800, von Humboldt had to face one of the classical problems of ethnopharmacology:

We are unable to make a botanical identification because this tree [which produces the raw material for the production of curare] only grows at quite some distance from Esmeralda and because [it] did not have flowers and fruit. I had mentioned this type of misfortune previously, that the most noteworthy plants cannot be examined by the traveller, while others whose chemical activities are not known [i.e. which are not used ethnobotanically] are found covered with thousands of flowers and fruit.

Later, the botanical source of curare was identified as *Chondrodendron tomentosum* Ruiz et Pavon, which produces the so-called tube curare (named because of the bamboo tubes used as storage containers). Other species of the Menispermaceae (*Chondrodendron* spp., *Curarea* spp., and *Abuta* spp.) and species of the Loganiaceae (*Strychnos* spp.) are also used in the production of curares.

The first systematic studies on the pharmacological effects were conducted by the French physiologist Claude Bernard (1813–78). It is worth looking at his description of the pharmacological effects of curare in some detail. “If curare is applied into a living tissue via an arrow or a poisoned instrument, it results in death more quickly if it gets into the blood vessels more rapidly. Therefore death occurs more rapidly if one uses dissolved curare instead of the dried toxin” (Bernard,25 p 92). “One of the facts noted by all those who reported on curare is the lack of toxicity of the poison in the gastrointestinal tract. The Indians indeed use curare as a poison and as a remedy for the stomach” (Bernard,25 p 93). Bernard was also able to demonstrate that the animals did not show any nervousness and any sign of pain. Instead, the main sign of death induced by curare is muscular paralysis. If the blood flow in the hind leg of a frog is interrupted using a ligature, but without interrupting the innervation, and it is poisoned via an injury of the hind leg, it retains its mobility and the animal does not die from curare poisoning (Bernard,25 p 115). These and subsequent studies allowed a detailed understanding of the pharmacological effects of curare in causing respiratory paralysis. The most important compound responsible for this activity was isolated for the first time from *C. tomentosum*, and in 1947 the structure of the bisbenzylisoquinoline alkaloid D-tubocurarine was established. Finally, tubocurarine’s structure was established unequivocally using nuclear magnetic resonance (NMR) in the 1970s, showing that it has only one quaternary nitrogen. In many European countries, tubocurarine is currently used only sporadically, but in France, for example, it is still used for muscle relaxation during surgery.

The use of medicinal plants was always an important part of all medical systems of the world, and Europe was no exception. Little is known about popular traditions in medieval and early modern Europe. Our knowledge starts with the availability of written (printed) records on medicinal plant use by common people. As pointed out by Griggs,26 a woman in the seventeenth century was a ‘superwoman’ capable of administering “any wholesome receipts or medicines for the good of the family’s health” (p 88). A typical case is foxglove (*Digitalis purpurea* L., Scrophulariaceae), reportedly used by an English housewife to treat dropsy, and then more systematically by the physician William Withering (1741–99). He transformed the orally transmitted knowledge of British herbalism into a form of medicine that could be used by medical doctors. Prior to that, herbalism was more of a clinical practice interested in the patient’s welfare, and less of a systematic study of the virtues and chemical properties of medicinal plants.

Below are listed examples of natural products first identified during the early years of the nineteenth century and briefly summarize information on subsequent research to fully characterize these compounds and to establish their structures. All these activities were automatically based on the common medical use of these species. Today, they would thus be considered ethnopharmacologically driven.

*Examples of pure compounds first isolated during the early nineteenth century:*

- 1804 – Morphine (1) from the opium poppy (*Papaver somniferum* L., Papaveraceae) was first identified by F. W. Sertürner (Germany). It took until 1817 for it to be chemically characterized as an alkaloid. Its structure was established in 1923 by J. M. Gulland and R. Robinson England.
• 1817 – Emetine from ipecacuanha (*Cephaelis ipecacuanha* (Brot.) A. Rich., Rubiaceae) was fully characterized as late as 1948 and used as an emetic as well as in cough medications
• 1817 – Strychnine from *Strychnos* spp., Loganiaceae, was used as a tonic and stimulant
• 1820 – Quinine (2) was first isolated from *Cinchona* spp. (Rubiaceae) by Pierre Joseph Pelletier and Joseph Bienaime Caventou of France: the structure was elucidated in the 1880s by various laboratories
• 1821 – Caffeine (3) from the coffee tree (*Coffea arabica* L. and *C. canephora* Pierre ex. Froehn, Rubiaceae); its structure was elucidated in 1882
• 1826 – Coniine, a highly poisonous natural product, was first isolated from hemlock (*Conium maculatum* L., Apiaceae). Its properties had been known for years (Socrates sentenced to death by drinking a mixture containing poison hemlock). It was the first alkaloid to have its structure elucidated (1870). Some years later, it was synthesized (1889)
• 1833: Atropine from belladonna (*Atropa belladonna* L., Solanaceae) used at the time for asthma; today, the compound is still used in ophthalmology
• 1846: L. Thresh isolated capsaicin from *Capsicum frutescens* L., s.l. Its structure was partly elucidated in 1919 by E.K. Nelson

(modified after Heinrich *et al.* based on Sneader and others)

Morphine, for example, derived from the opium poppy (*P. somniferum*, Papaveraceae), was first identified by F. W. Sertürner (Germany) in 1804 and first chemically characterized in 1817 as an alkaloid. Its structure was finally established in 1923 by J. M. Gulland and R. Robinson in Manchester. There can be no doubt that this development was driven by local and traditional knowledge. The opium poppy was and is still used widely as both a medicine and a recreational drug of abuse. The opium poppy (family Papaveraceae) is an annual plant native to Asia. It is cultivated widely for food (the seed and seed oil), for medicinal purposes, and as a garden ornamental. It has been used since time immemorial as a painkiller, sedative, cough suppressant, and antidiarrheal and is featured in ancient medical texts, myths, and histories.

Quinine from Cinchona bark (*Cinchona pubescens* Vahl. and others) was first isolated by Pierre Joseph Pelletier and Joseph Bienaime Caventou of France in 1820 and the structure was elucidated in the 1880s by various laboratories. These two researchers were also instrumental in isolating many of the alkaloids listed above.

Salicin, from willow bark (*Salix* spp., Salicaceae), was first isolated by Johannes Buchner in Germany. It was derivatized first to yield salicylic acid (1838, Rafaele Pirea, France) and later, by the company Bayer in 1899, to yield acetyl salicylic acid, or aspirin – a compound previously known but which had not been studied pharmaceutically.

### 3.12.2.2 The First Half of the Twentieth Century
#### 3.12.2.2.1 Antibiotics as a new model
Penicillin was further developed by Howard Florey and Ernst Chain in the late 1930s. One of the most important events that influenced the use of ethnopharmacology-driven drug development in the last century was the serendipitous discovery of the antibacterial properties of fungal metabolites such as benzylpenicillin by Alexander Fleming in 1928 at St. Mary’s Hospital (London, Paddington). These natural products changed...
forever the perception and use of plant-derived metabolites as medicines by both scientists and the lay public.27 From this point onward, in terms of drug discovery, plant-derived drug leads, generally based on local and traditional knowledge, competed with the chemosystematic diversity of microorganisms. This diversity resulted in tremendous discoveries most importantly as anti-infective agents. Clearly, and with only a few exceptions, microorganism-based drug discovery cannot be ethnopharmacologically driven.

Another important development came with the advent of synthetic chemistry in the field of pharmacy. Many of these studies involved compounds that were synthesized because of their potential as coloring materials.28 The first successful use of a synthetic compound as a chemotherapeutic agent was achieved by Paul Ehrlich in Germany (1854–1915), who used methylene blue in the treatment of mild forms of malaria in 1891. Unfortunately, this finding could not be extended to the more severe forms of malaria common in the tropics. Many further studies on the therapeutic properties of dyes and of other synthetic compounds followed. The later twentieth century also saw a rapid expansion in the knowledge of secondary natural products, their biosynthesis, and their biological and pharmacological effects. There is now a better understanding of the genetic basis of the reactions that give rise to such compounds, and also the biochemical (and in many cases genetic) basis of many important illnesses. This has opened up new opportunities and avenues for drug development.

This is important in the context of our discussion here because it highlights the fact that during this period alternative strategies offered novel ways to discover and develop new drugs and drug leads. Serendipity and more random approaches ultimately led to a strategy where the essential goal was an increase in the total number of samples to be screened, resulting in high-throughput technologies.

3.12.2.3 Do We Need Ethnopharmacology-Driven Drug Development? 1945 Until the 1990s

3.12.2.3.1 Compounds with an effect on the central nervous system

One of the most famous examples of a drug development project driven by traditional knowledge is the discovery of psilocybin and derivates from the hallucinogenic mushroom *Psilocybe*, which for centuries has been used by the Mazatec Indians in Oaxaca, Mexico. This drug development project of the 1940s and 1950s was only possible thanks to the collaboration of two ethnobotanists and two chemists. R. G. Wasson (1898–86) had been trained as a journalist and in literature studies. Thanks to his wife Valentina Pavlovna Guercken, he became interested in ethnobotany. This brought him in contact with the American ethnobotanist Richard Evans Schultes (1916–01), who, while doing his Ph.D. dissertation in the Mazatec region, learned about the use of hallucinogenic mushrooms commonly known by the Aztec name ‘teonanacatl’. While continuing to work they devoted much of their spare time to the study of these ‘enthogens’. R. G. Wasson ultimately became the first outsider to participate in a nightlong *velada*, a ‘stay-awake’ in the community of Huautla de Jimenez, Mexico. These experiences were publicized very widely and in 1957 they were even reported in detail in *Life* magazine.

![Psilocybin](image1)

![LSD](image2)

The last two persons who were involved in the discovery of the new leads were Swiss chemist Albert Hofmann (1906–08) and natural product chemist Robert F. Raffauf (1916–01). Phytochemical studies indicated that the pharmacological activity is due to relatively simple alkaloids, especially psilocybin (4), which is a phosphate salt in the fungi, and the *in vivo* active metabolite psilocin. Hofmann developed a semisynthetic derivative – lysergic acid diethylamide (LSD) (5), which was to be developed as a psychoactive medication and
which also shows structural similarities to the ergot alkaloids. The compound is structurally also closely related
to other indole alkaloids like ergotamine from the sclerotia of *Claviceps purpurea* (ergot), a compound also
developed on the basis of local (European) knowledge. The expectations for developing new drugs based on
this ethnomycological information were ultimately not met, but the compound became one of the most
problematic drugs of abuse. The species that yield these compounds are Popularly used as mind-altering
drugs (e.g., *Lophophora williamsii* (Lem. ex Salm-Dyck) Coult., a Cactaceae, and the ‘magic mushrooms’ (*Psilocybe*
and related genera) discussed above). In regions of study, drastic sociocultural changes were the result of these
research projects, especially because of the popularization of this sacred and specialized information and the
subsequent influx of nonnatives.

Galanthamine (syn. galantamine, 6) is a natural product known from several members of the amaryllis family
(*Amaryllidaceae*) and the idea for developing a natural product from these species seems to be based on the
local use of one of these species in a remote part of Europe (ethnobotanical information). Today, galantha-
mine (esp. under its brand names Reminyl and Nivalin) is commonly used in the treatment of Alzheimer’s
disease. This example highlights both the uncertainties and problems of linking information about local and
traditional uses with a compound’s development. Broadly, speaking the development of galanthamine into a
widely used Alzheimer’s drug can be divided into three main periods:

- Early, development in Eastern Europe for use in the treatment of poliomyelitis
- Preclinical, development in the 1980s into an Alzheimer’s medication
- Clinical, development in the 1990s

In the context of this review, the first phase is of particular relevance. The early development of galanthamine
in Eastern Europe for use in the treatment of poliomyelitis started with the alkaloid’s isolation from the garden
snowdrop (*Galanthus* spp., most notably *G. woronowii*), but today the compound is obtained from other members
of the same plant family like the daffodil (*Narcissus* spp.) and the snowflake (*Leucojum* spp., esp. *L. aestivum*) as
well as being made synthetically.

*Galanthus* species are native to many parts of Europe including Bulgaria, the eastern parts of Turkey, and the
Caucasus mountain range. Overall, little is known about the local and traditional uses of this genus in Europe.
A. Plaitakis and R. C. Duvoisin hypothesize that Homer’s ‘moly’ might have been the snowdrop, *Galanthus nivalis*. In his epic poem the Odyssey, he described ‘moly’ and its use by Odysseus as an antidote against Circe’s
poisonous drugs. Thus the description of ‘moly’ as an antidote in Homer’s Odyssey may represent the oldest
recorded use of *Galanthus*, but the evidence is scanty. The ‘classical’ medicobotanical texts of the sixteenth
century (i.e., Fuchs, Bock, and Brunfels) do not mention the snowdrop (*G. nivalis*) and make only cursory
reference to *Leucojum*. Interestingly, the German pharmacognosist G. Madaus does not mention *Galanthus* or
*Leucojum* and only discusses *Narcissus pseudonarcissus*, giving some isolated uses that have no direct association
with the Central nervous system (CNS), whereas Marzell does not discuss any of the three genera. In
F. Köhler ‘Arzneipflanzen’, practically no medical use is given for species of the three genera. Thus, it is
certain that *Galanthus* and other genera of the *Amaryllidaceae* were not commonly used European medicines.
On the contrary, this clearly does not exclude local and traditional uses in rural regions of Europe and Asia.

According to unconfirmed reports, in the 1950s, a Bulgarian pharmacologist noticed the use of the common
snowdrop growing in the wild by rubbing on their foreheads to ease nerve pain. Also, some of the earlier
publications indicate extensive use of snowdrop in Eastern Europe, such as Romania, Ukraine, Balkan
Peninsula, and Eastern Mediterranean countries. However, we were unable to trace down any relevant
ethnobotanical literature. In the first pharmacological publication on galanthamine, no reference is made to the traditional use of snowdrop in the Caucasian region by the Russian authors. An interesting note comes from the London pharmacognosist E. J. Shellard and was published as a letter to the editor of the *Pharmaceutical Journal* (UK): He recalls a presentation in 1965 by “a Russian pharmacognosist reporting about a peasant woman living at the foot of the Caucasian mountains (Southern Russia, Georgia) who, when their young children developed symptoms of an illness which, as he described them, was obviously poliomyelitis, they gave them a decoction of the bulbs of the Caucasian snowdrop (*Galanthus woronowii* Los) [sic] and the children completely recovered without showing any signs of paralysis”. This is one of the few, secondhand reports currently available recording the use of snowdrop prior to the development of galanthamine as a licensed medicine (see Table 1). Systematic exploration by the author with colleagues from central Europe and Russia resulted in one additional, but still secondhand review. According to Teodora Ivanova of the Bulgarian Academy of Sciences (personal communication, 2008), an alcoholic extract of *L. aestivum* L. was used by her grandparents and other older people in the eastern parts of Bulgaria. The extract was reported to be used in the prevention or treatment of memory loss, but because this record postdates the introduction of galanthamine as an Alzheimer’s medication onto the worldwide market, this report may not actually be a secondary outcome of the species’ use to extract galanthamine for clinical use.

Most of the early investigation on galanthamine was conducted in Bulgaria and the USSR during the coldest period of the Cold War. In the early 1950s, the Russian pharmacologist Mashkovsky worked with galanthamine isolated from *G. woronowii*. In 1951, M. D. Mashkovsky and R. P. Kruglikova-Lvov used an *ex vivo* system (rat smooth muscle) to prove its acetylcholine esterase (AChE)-inhibiting properties. Consequently, this is the first published work that proves AChE-inhibiting properties of galanthamine. In 1952, N. F. Proskurnina and A. P. Yakovleva established and published the chemical structure of galanthamine as an alkaloid with a tertiary nitrogen atom, again based on material isolated from *G. woronowii*. Also, the compound’s physicochemical characteristics were determined. In 1955, Mashkovsky published a second paper on the

### Table 1 Historical development of galanthamine as a clinically used drug

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<th>Year</th>
<th>Development step of galanthamine</th>
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<tr>
<td>Early 1950s</td>
<td>Russian pharmacologist discovers that local villagers living at the foot of the Ural mountains use wild Caucasian snowdrop to treat (what he considers to be) poliomyelitis in children</td>
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<td>1952</td>
<td>Galanthamine was first isolated from <em>G. woronowii</em></td>
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<td>1956</td>
<td>D. Paskov suggested that galanthamine can be extracted from the leaves of <em>Galanthus</em></td>
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<td>Late 1950s</td>
<td>Various preclinical studies on the pharmacology of galanthamine were carried out. For instance,</td>
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<td>i. Galanthamine was found to have antagonistic effects against nondepolarizing neuromuscular blocking agents. This has been shown in experiments on neuromuscular preparation of cats <em>in situ</em>, in experiments <em>in vitro</em> on frog rectus abdominis muscle, etc.</td>
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<td></td>
<td>ii. <em>In vivo</em> and <em>in vitro</em> experiments were done in rats for determining the effects of galanthamine on the brain</td>
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<td>Galanthamine was registered as a medicine under the trade name ‘Nivalin’ and is commercially available in Bulgaria</td>
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<tr>
<td>Early 1960s</td>
<td>The first data on anticholinesterase activity of galanthamine was reported from an <em>in vivo</em> study in an anesthetized cat</td>
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<tr>
<td>1980s</td>
<td>Preclinical development: Researchers searching for novel treatments of Alzheimer’s disease started investigating the therapeutic effects of galanthamine</td>
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<td>1990s</td>
<td>Clinical development of galanthamine into a medication for Alzheimer’s disease</td>
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<td>1996</td>
<td>Sanochemia Pharmaceutika obtained the first patent on the synthetic process of galanthamine</td>
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<td>1997</td>
<td>Sanochemia began collaboration with a Belgium-based company (Janssen Pharmaceutica) and an emerging British company (Shire Pharmaceuticals Group plc)</td>
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<tr>
<td>2000</td>
<td>Galanthamine licensed in the first countries (Iceland, Ireland, Sweden, UK) for the treatment of Alzheimer’s disease</td>
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<td>Currently</td>
<td>Galanthamine has been approved for use in the United States, many European countries, and many Asian countries. Controversies remain over the therapeutic benefits of acetylcholinesterase inhibitors, since they delay the onset of more severe symptoms and offer no curative treatment</td>
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Adapted from M. Heinrich; H. L. Teoh, *J. Ethnopharmacol*. 2004, 92, 147–162.
cholinesterase-inhibiting properties of galanthamine. Unfortunately, Mashkovsky does not indicate the source of the galanthamine used, but most probably Mashkovsky worked again with galanthamine isolated from \textit{G. woronowii}. In 1956, the Bulgarian pharmacologist D. Paskov discovered galanthamine in the European daffodil and in the common snowdrop, \textit{G. nivalis}. Paskov suggested extracting galanthamine from the leaves of \textit{G. nivalis}. In 1957, this scientist, who trained in Russia under Mashkovsky, published his results from the study of \textit{L. aestivalum} (summer snowflake) and its content of galanthamine, which was to become the main source of the compound. In 1960, a full chemical synthesis was published. This was a biomimetic laboratory process with a yield below 1% and had been designed as proof of structure, not for industrial production.

The indication polyomyelitis, which was the main indication in the Eastern Block from 1950 until a few years ago, came as a result of the data that galanthamine enhances nerve impulses transmission at the synapses. In the form of hydrobromide salt, it became commercially available as a registered product under the trade name ‘Nivalin’. Furthermore, galanthamine shows extremely potent antagonizing action against curare (\textit{d}-tubocurarin; Nikolev, personal communication, 2003).

Many preclinical studies were carried out in animals for testing the pharmacological activity of galanthamine. After a few years, some researchers demonstrated the penetration of galanthamine through the blood–brain barrier, and thus effects on the CNS became of particular interest. Based on the knowledge of galanthamine in both peripheral nervous system and CNS, many countries in Eastern Europe had used it as an acknowledged treatment in myasthenia gravis and muscular dystrophy, residual poliomyelitis paralysis symptoms, trigeminal neurologia, and other forms of neuritides.

Overall, this is not only an example of the successful ethnobotany-driven development of a natural product into a clinically important drug, but also highlights that it is often difficult to establish the link between local and traditional uses and drug development. Ethnobotany gave an essential, initial hint, but at this point the evidence where the initial ethnobotanical information comes from remains scanty.

A second case relates to a pharmaceutical product that in many countries is not considered to be a medicine, while in others it has been one of the best selling herbal medicines – a special extract obtained from the leaves of \textit{Ginkgo biloba} L. The most important use of \textit{Ginkgo} is in age-related disorders. It is especially used to prevent or reduce memory deterioration and milder forms of dementia including the early stages of Alzheimer’s disease. It enhances cognitive processes, and experimental evidence points to improvements in blood circulation to the brain and anti-inflammatory and antioxidant effects.

The species is a living fossil and has survived in China, where it is found mainly in monasteries in the mountains and in palace or temple gardens. In Asia, \textit{Ginkgo} is an object of veneration, and is considered a sacred tree of the East; it has been seen by some as a symbol of changelessness, possessing miraculous power, bearer of hope and of the immeasurable past, a symbol of love, and unity of opposites. Because of all its properties, it is associated with longevity. Buddhist monks cultivated the tree from about AD 1100 for its many good qualities. It was spread by seed to Japan (around AD 1192, associated with Buddhism) and Korea. In the oldest Chinese literature, \textit{Ginkgo} is not mentioned, but in the eleventh century (Sung dynasty) it appeared in the literature as a plant native to Eastern China. When \textit{Ginkgo} was transplanted in the residence of Prince Li Wen-ho in the first half of the eleventh century, came from the south and by transplanting it in his residence, it became famous and spread through propagation. From that time on, \textit{Ginkgo} has been depicted in Chinese paintings and appeared in poetry. Scientists thought that \textit{Ginkgo} had become extinct, but in 1691, Engelbert Kaempfer, a German naturalist, discovered \textit{G. biloba} trees in Japan, and in 1730 it was brought to Europe (Utrecht).

The earliest known medicinal use dates back to 2800 BC and is described as the pseudofruits of \textit{G. biloba}. There are many historic and modern medical uses of the pseudofruit. Interestingly, the leaves are much less frequently used in Eastern Asia. One use is to treat chilblains (reddening, swelling, and itching of the skin due to frostbite) and as a throat spray for asthma. Europeans were fascinated by this tree since they first discovered it because it symbolizes longevity and its leaves have a unique structure. It fascinated poets and scientists alike, including the famous German poet and natural historian J. W. von Goethe:

\begin{verbatim}
This leaf from a tree in the East,
Has been given to my garden.
It reveals a certain secret,
Which pleases me and thoughtful people.
\end{verbatim}
Does it represent One living creature
Which has divided itself?
Or are these Two, which have decided,
That they should be as One?
To reply to such a Question,
I found the right answer:
Do you notice in my songs and verses
That I am One and Two?38

Ginkgo contains two major types of pharmacologically active constituents – diterpene lactones, for example, ginkgolides A, B, and C and bilobalide, as well as flavonoids, the most important being the biflavone glycosides such as ginkgetin, isoginkgetin, and bilobetin, which also contribute to its activity. Ginkgolic acids are present in the fruit but normally only in very minor amounts in the leaf. Based on some not very well documented uses in traditional Chinese medicine (TCM), a German company, Dr. Willmar Schwabe Pharmaceuticals, first developed a poorly characterized ethanolic and later a 'special' extract – extract G. biloba (EGb) 761 – which is based on an ethyl acetate extraction and subsequent fractionation. The extract was developed into a highly successful phytomedicine. Unfortunately, the history of development of this extract is not well documented, and little information seems to be available within the company. Initial research in the mid-1960s identified flavonoid glycosides as active constituents of G. biloba leaf extracts. In 1971, the first patent on the complete extraction and standardization was filed in Germany and a year later in France.39 These patents describe the process for obtaining a ‘mixture of vasoactive substances’ and formed the basis for the highly successful clinical development for the indications listed above.

This example is of interest, because it highlights that the symbolic importance both in Asian and European countries was a driving force for developing this into a medication. There may not have been a direct link between the traditional use and modern European medical use, but species association with longevity presumably has provided the ideas for pharmacological experiments, which ultimately resulted in the development of a ‘rational phytomedicine’.40 Also, this example is the first one that highlights the development of a standardized extract for use as a medicine based on traditional knowledge systems (in this case, TCM) into an over-the-counter herbal medical product. In later years, numerous similar development projects resulted in novel phytomedicines including Hypericum perforatum L. (St. John's Wort, Hypericaceae) used for mild to moderate depression, Harpagophytum procumbens (Burch) DC. (Devil's Claw, Pedaliaceae) used for chronic pain, and Piper methysticum G. Forst. (kava kava, Piperaceae) for relieving anxiety. P. methysticum, for example, originates from many Pacific islands. Best known is its religious and/or symbolic use.41 It is consumed under very strict sociocultural control. On many islands, for example, the local leader is the only or at least the first one to drink it. It is often prepared by chewing the root and rootstock and then spitting the mixture into a large bowl. According to local Pacific traditions, P. methysticum is the ideal species to overcome social tensions and to help to (re-)establish proper social relations. This offered a clear and direct lead for developing a phytomedicine, which for many decades, but especially since the 1960s, has been used as a mild stimulant and has been a widely acclaimed treatment for this condition. However, in 2001, kava kava-containing drugs were withdrawn from practically all markets due to suspected hepatotoxic effects. In this therapeutic area as in many other areas, ethnopharmacology-driven drug development continues to be an exciting opportunity. Recently, over 150 plant species in various preparations and mixtures with the potential for R&D on developing new drug leads for age-related cognitive disorders were found by systematically assessing the information available in Swiss university libraries.42

3.12.2.3.2 Anticancer agents developed between 1950 and 1980
Etoposide (Vepesid, 8) and teniposide (VM-26, 9) are well-known topomerase II inhibitors. Both are semisynthetic derivatives of podophyllotoxin first isolated from Podophyllum peltatum L., a native American remedy for warts, and is used as a purgative. Ethanolic extracts of the rhizomes are known as Resina podophylli (podophyllin). This resin was included in many pharmacopoeias for the topical treatment of warts and benign tumors (condylomata acuminate) (and as Podophyllum Resin is still included in some pharmacopoeias like the British Pharmacopoeia).43 It is highly irritating and unpleasant and therefore can only be used topically.
Podophyllotoxin (7) was first isolated in 1880 and its structure was proposed in 1932. Clearly, this usage was one of the reasons for the species’ selection for anticancer screens. This natural product is also found in other Podophyllum species like P. brevifolia Royle (syn. P. emodi, Berberidaceae) from India and China.

The second case is the vinca alkaloids – vinblastine (10), vincristine (11), and navelbine (12) – from Catharanthus roseus (L.) G. Don (formerly called Vinca rosea, Madagascar periwinkle, Apocynaceae). As the name indicates, the species is originally from Madagascar, but researchers at the National Cancer Institute (NCI) of the United States actually worked with samples collected in the Caribbean, where the plant was used locally to treat diabetes. By the early twentieth century, it was used as an oral hypoglycemic agent (to lower blood sugar levels) in South Africa, Southern Europe, and the Philippines to treat diabetic ulcers in the British West Indies and in Brazil to control hemorrhages and scurvy. It was the role of the plant as an antidiabetic agent in the Caribbean that led to the discovery of its effective anticancer activity. In 1952, a patient from Jamaica sent a sample of the plant to Dr. Clark Noble, a Canadian researcher, who forwarded it to his brother Dr. Robert L. Noble (at the University of Western Ontario) and Dr. J. B. Collip, researchers who helped refine insulin. This prompted a small scientific study, which found that rats given tea, which was made from crushed Madagascar periwinkle from which ‘vinblastine’ was isolated, had a significantly lowered white blood cell count. Although this mixture was fatal to the rats, this action prompted the interest of the researchers to assess the action of the Madagascar periwinkle against leukemia – a disease caused by an abnormal increase in white blood cells, first reported in 1958. The active principle was identified vinblastine, a new alkaloidal compound. Vinblastine was licensed in the United States and approved for use in cancer treatments in 1961. Prior to this, industrial processes for isolation had to be developed, a task taken on by Eli Lilly & Co. under the scientific leadership of the chemist Gordon Svoboda and collaborators, who were also instrumental in identifying a related alkaloid from the same plant, vincristine, which was licensed as a drug 2 years later. Vince alkaloids bind to β-tubulin and inhibit microtubule assembly. Vindesine and vinorelbine are novel vinca alkaloid derivatives with improved clinical features for tumor therapy.

The previous example highlights how difficult it is to establish retrospectively whether a compound has had local and traditional uses and specifically whether vinca alkaloids are directly linked to the therapeutic uses of the compound in biomedicine. The most recent clinically significant discovery from the NCI screening program is taxol (13), from Taxus brevifolia Nutt. (Taxaceae). It has been argued many times that this discovery was not ethnomedicine driven, but considerable evidence highlights the importance of T. brevifolia in Native American medicine. Even though the initial sample was collected as part of a random sampling approach, T. brevifolia has been reported to be used by a variety of western Indian groups (USA and Canada) as a medicine and also for producing a variety of useful products (canoes, brooms, combs). Very diverse ethnomedical uses of the root and the bark are recorded and include several reports for stomachache and only in case of the Tsimshian tribe (British Columbia, Canada) in the treatment of cancer. Thus, unknown to researchers, the Tsimshian selected a plant with a high cultural salience in many western North American cultures. This example highlights the fact that species used to isolate medicines are highly likely to have traditional uses.

It showed activity in the NCI's cancer screening platform, and the core compound taxol was first isolated in the mid-1960s by Monroe Wall (1916–2002), Mansukhlal C. Wani, and coworkers. After some initial research, the project was halted in 1971. In 1977, its activity against a melanoma cell line and in the human xenograft model led to the start of preclinical development. Initially, there were problems in acquiring large amounts of the compound, but solutions to these problems and the report of taxol’s unique mode of action by promotion of tubulin polymerization and stabilization of microtubules against depolymerization increased the interest. Clinical studies started in 1984. Prior to this, studies on the compound's toxicology and the pharmacological mechanism of action were conducted. It took a further 10 years before taxol was approved by the FDA in the treatment of anthracyclin-resistant, metastasis-forming breast cancers. Taxol has excellent activity against ovarian and breast cancers, but it also has serious side effects. In the meantime, the compound has been approved for a variety of other cancers and now semisynthetic derivatives are also employed. Although it is generally considered to be a metabolite of Taxus sp. and associated endophytic fungi, taxol was also found in shells and leaves as well as in cell cultures of Corylus avellana L. (the hazelnut shrub, Betulaceae). In addition to taxol, 10-deacetyl-baccatin III, baccatin III, paclitaxel C, and 7-epipaclitaxel were also identified and quantified in shells and leaves. The finding of these compounds in shells, which often are waste products of mass production in the food industries, may open new avenues of supply for this anticancer agent.
Even though the initial sample was collected as part of a random sampling approach, local and traditional uses clearly predate the R&D activities of the NCI and associated researchers. The fact that the local and traditional knowledge on *T. brevifolia* was not known to these researchers may indicate that it is the outcome of a random screen, but clearly the fact that ethnopharmacologically preselected species were developed highlights that such local and traditional knowledge is an excellent starting point for drug development.

*Camptotheca acuminata* Decne (Xi Shu, tree of joy, Nyssaceae) is widely used in TCM and, therefore, was included in 1958 in a screening program at the NCI where it gave positive results. Wood and bark (20 kg) were collected for extraction; These extracts were shown to be active against a mouse leukemia life prolongation assay in which it was unusual to find activity. The fractionation and anticancer testing was a very slow process and finally resulted in the isolation and structure elucidation (in 1966) of camptothecan (15), a highly unsaturated quinoline alkaloid with a unique (at the time) structure as an α-hydroxylactone. *C. acuminata* was shown to be extremely active in the life prolongation assay of mice treated with leukemia cells and in solid tumor inhibition. These activities encouraged the NCI to initiate clinical trials with the water-soluble sodium salt. While the results of some studies conducted in the United States were disappointing, in a clinical trial in China with 1000 patients the sodium salt showed promising results, for example, against head, neck, gastric, intestinal, and bladder carcinomas.28

As these examples show, the taxanes (*taxol*, 13, and *taxotere*, 14), agents derived from podophyllotoxin (etoposide and teniposide), the vinca alkaloids (*vinblastine*, 10, and *navelbine*, 12), and the camptothecine (15)-derived anticancer agents (*topotecan*, 16, and *irinotecan*, 17) all exemplify a similar situation. The drugs, which yielded the anticancer agents (and ultimately their derivatives), were all important medicines in their respective cultures. Although this may have not been recognized at the time of initial discovery, it is an astounding fact that all species of plants have a tradition of medical use. Researchers may not have known it at the time of their research, but they followed a path healers in the various cultures had taken many generations before them.

In recent years, more direct benefits for the providers (the states and their peoples) have become a core element of discussion. Ethnobiological research and any other research involving the use of biological resources of a country are today based on agreements and permits, which in turn are based on international and bilateral treaties. The most important of these is the Convention of Rio or the Convention on Biological Diversity (CBD),\(^{51}\) which looks at the rights and tasks associated with biodiversity at an international level:

The objectives of this Convention, to be pursued in accordance with its relevant provisions, are the conservation of biological diversity, the sustainable use of its components and the fair and equitable sharing of the benefits arising out of the utilisation of genetic resources, including by appropriate access to genetic resources and by appropriate transfer of relevant technologies, taking into account all rights over those resources and to technologies, and by appropriate funding.
The rights of indigenous peoples and other keepers of local knowledge is addressed in article 8j:

(j) Subject to its national legislation, respect, preserve and maintain knowledge, innovations and practices of indigenous and local communities embodying traditional lifestyles relevant for the conservation and sustainable use of biological diversity and promote their wider application with the approval and involvement of the holders of such knowledge, innovations and practices and encourage the equitable sharing of the benefits arising from the utilization of such knowledge, innovations and practices.

This and the subsequent treaties significantly changed the basic conditions for ethnopharmacological research. Countries that provide resources for natural product research and drug development have well-defined rights, which specifically includes sharing benefits that may potentially arise from such research.

Especially in case of ethnopharmacological research, the needs and interests of the populations a researcher is collaborating with also become an essential part of the research. As pointed out many times, 'there is an inextricable link between cultural and biological diversity'. This principle was first formulated at the First International Congress on Ethnobiology in Belem in the year 1988. No generally agreed upon standards have so far been accepted, but the importance of obtaining the informants’ prior informed consent and ascertaining appropriate benefit-sharing agreements has been stressed by numerous authors (e.g., Posey52), even though the exact requirements of such arrangements sometimes remain contentious.

Numerous other agreements (like TRIPS (trade-related aspects of intellectual property rights), WTO (World Trade Organization) agreements, cf. www.wto.org) are also of relevance, but it is beyond the scope of this chapter to address the complexity of national and international agreements.

3.12.2.5 The Revolution of Molecular Biology: From the 1990s Until Today

The previous examples (Sections 3.12.2.1–3.12.2.3) also highlight the shift from organism- or cell-based screening system, which was the mainstay of drug development until about the 1980s, to a more biochemical–mechanistic approach. This chapter highlights projects that have come into fruition in the last years and that extensively use modern molecular–biological approaches. Also, these examples emphasize the central role of the Convention of Biological Diversity and related agreements in the drug discovery and development process.

3.12.2.5.1 Antiparasitic and insecticidal agents

Quinine has been one of the first biologically active natural products to have been isolated and has had a tremendous impact on drug development programs (see above). Similarly, the discovery of artemisinin and its analogues as potent antimalarial agents has been among the prime examples of ethnopharmacology-driven drug discovery. Recently, the alkaloid cryptolepine from the west African Cryptolepis sanguinolenta (Lindl.) Schltr., used traditionally in the treatment of malaria, has received considerable attention. In 2005, these examples were reviewed by C. W. Wright.53 This is an area of drug discovery where direct ethnopharmacological links have been well documented. For hundreds of years, *Artemisia annua* L. (Asteraceae, Qing Hao) has been used in TCM. The leaves were harvested in the summer, before the plant comes into flower, and dried for later use. It is generally used in the treatment of fever, malaria, colds, diarrhea, as a digestive, and, externally, as a vulnerary.

*Artemisia annua* has been known since the Zhou Hou Bei Ji Fang – (Handbook of Prescriptions for Emergency Treatment) of Ge Hong of AD 340 as a treatment for fevers. In 1967, a group of Chinese scientists started a search for new antimalarial drugs from Chinese medicine. Only in 1977 did a Chinese research group isolate the active principle, the sesquiterpene lactone artemisinin,54 which proved to be very potent against the malarial parasite *Plasmodium falciparum* and especially against chloroquine-resistant malaria.55 The development of this sesquiterpene lactone with a highly unusual endoperoxide moiety was based directly on traditional and local knowledge. Clinical trials in China in a large number of patients showed that artemisinin (18) was highly effective in clearing parasitemia and reducing symptoms in patients with malaria, including some with chloroquine-resistant malaria and/or cerebral malaria.53 However, for many years, lack of funding was a major problem in this area (see below). Interestingly, the compound also shows considerable promise as an
anticancer agent. In an attempt to overcome the problem of the recrudescence (1 month after the treatment, many patients have a recurrence of the illness), a number of derivatives of artemisinin (18) have been developed (ethers, such as artemether and arteether, and esters, such as sodium artesunate and sodium artenlinate). Although the compound is used as a first-line treatment, combination therapies are generally considered to be the best available choice. One core problem that has plagued the treatment of tropical diseases remains the limited access of the poor to such effective treatments and a continuous lack of funding for natural product-based drug development. However, such locally based drug development projects would also offer unique advantages once the results of preclinical and clinical work were implemented locally.

Although it was not developed based on the concepts of molecular biology, Azadirachta indica A. Juss. (syn. Melia azadirachta, Antelaea azadirachta), or neem, has become a classical case of a drug development process rife with controversies regarding the species’ traditional use. It is a principal species used within Indian Ayurvedic medical traditions and today is a pan(sub-)tropically grown tree. Neem is thought to have originated in the northeastern region of India (Assam) and in Burma/Myanmar. The exact location of origin is uncertain. It has been attributed to the entire Indian subcontinent and others to dry forest regions throughout all South and Southeast Asia, including Pakistan, Sri Lanka, Thailand, Malaysia, and Indonesia. The introduction of neem to East Africa is thought to have arisen during the construction of the Kenya–Uganda railways. Indian migrant workers are believed to have brought neem seed with them in order to cultivate this important medicinal plant.

The species is drought resistant and thrives in arid conditions with an annual rainfall between 400 and 1200 mm. It can grow between 0 and 1500 m above sea level but is intolerant to freezing, extended periods of cold, and waterlogged soils. Neem trees can reach a height of 25–30 m and provide valuable shade with its dense canopy of pinnate leaves. Consequently, it is a species that has become planted or naturalized in many countries.

The neem tree possesses a kaleidoscope of medicinal uses that are found in all parts of the plant. As part of Ayurvedic medicine, the leaves (5–10) are chewed for 15 days in late winter in order to maintain a healthy body. Tonics prepared by boiling the leaves, often with other herbal constituents, are useful against intestinal worms, fevers, and internal ulcers. Externally, the juice of the leaves is applied to the skin for the treatment of boils and eczema. The twigs are used extensively in dental hygiene to brush the teeth and incorporated into pastes or mouth washes for sale on markets. Neem fruits are used against leprosy, intestinal worms, and urinary diseases. Neem oil (Margosa) is a chemically diverse mixture that includes the isoprenoid azadirachtin and a complex mixture called nimbidin (which contains nimbin) plus numerous fatty acids such as oleic and palmitic acids. The oil is used for chronic skin complaints, leprosy, and ulcers; it is commercially marketed as a natural botanical insecticide. Azadirachtin is the main insecticidal ingredient of neem.

Many controversies surround the development of this traditional insecticide and medicine: In 1992, the U.S. company W. R. Grace applied for a patent to extract seeds of the neem tree in a simple manner. The plant material is extracted with a lipophilic solvent (e.g., ethyl ether) instead of with a watery one, as it has been done for many centuries in India, resulting in an increased stability. However, is this really an innovation? American patent law does not recognize oral traditions like the Indian ones and approval of such a patent would have, for example, resulted in the exclusion of Indian companies from the U.S. market. This patent and some related ones have been revoked, but the overall conflict continues.
3.12.2.5.2 Antiviral and anticancer agents

Peplin Ltd. in Queensland, Australia, currently manufactures ingenol 3-angelate (or PEP005; 21), an unusual diterpene ester isolated from *Euphorbia peplus* L. (Euphorbiaceae) or petty spurge/radium weed/cancer weed. Most advanced are studies on the topical use for treating actinic keratoses and nonmelanoma skin cancer. In addition, it was developed for intravesicular treatment of bladder cancer systemically against leukemia. *E. peplus* was widely used in Europe and Morocco to treat warts and other skin conditions. The species was introduced into Australia and many other temperate countries. During the 1970s and 1980s, members of the Australian public used the sap from *E. peplus* to treat skin cancers and solar keratoses. A. C. Green and G. L. Beardmore reported that in Brisbane, Australia, *E. peplus* is the second most commonly used plant product treating these conditions. Only *Aloe vera* was used more frequently (35 reports). Overall, there were 164 persons (out of 2095 respondents) who indicated that they self-treated for skin cancers and solar keratoses. Of these, 75 used herbal medicines, whereas 8 used *E. peplus*. Another commonly used treatment was *Carica papaya* (8 reports). Although this is a relatively small number, it clearly served as a starting point to investigate the species' medical effects, proving that this R&D project was clearly ethnopharmacologically driven. Ingenol 3-angelate (PEP005) had an initial LD<sub>90</sub> of 180–220 \(\mu\text{mol l}^{-1}\) against a range of human and mouse cell lines. In vivo experiments using various tumors transplanted into mice indicated that a topical application for 3 days of 42 nmol formulated as an isopropanol-based gel was the most effective. The compound induced an acute erythema. Mechanistic studies indicated a rapid disruption of the plasma membrane, swelling of mitochondria, and cell death via primary necrosis. Experimental evidence exists that, at a second stage, neutrophil-mediated antibody-dependent cellular toxicity plays an important role. In vitro, ingenol 3-angelate has potent antileukemic effects in a large number of cell lines, inducing apoptosis in myeloid leukemia cell lines and primary acute myeloid leukemia cells at nanomolar concentrations. It was then established that this activity is correlated with the expression of PKC-\(\delta\) (protein kinase \(\delta\)). Interestingly, it induced a translocation pattern of PKC-\(\delta\) different from that of the well-known tumor copromoter PMA (phorbol 12-myristate-13-acetate (also known as PTA)). At low concentrations (10 nmol ml\(^{-1}\)), ingenol 3-angelate induces a rapid translocation of PKC-\(\delta\) simultaneously to the internal membranes and the nuclear membranes. PMA, on the contrary, causes PKC-\(\delta\) first to translocate to the plasma membrane and then to the nuclear membrane. In addition, ingenol 3-angelate modulates the activity of targets in the nuclear factor kappaB (NF-\(\kappa\)B) pathway. This activity is complex and time dependent. Up to 6 h after application of ingenol 3-angelate, a biphasic activation of p65 and, to a lesser degree, C-Rel, was observed. As of 2008, phase III clinical trials of topical use are planned.

This example offers some amazing insights into the complexity of modern drug discovery, especially as it relates to the ethnopharmacological links of the research. Without doubt, this discovery was driven by local and traditional knowledge. It is based on European ‘indigenous’ knowledge, which clearly had been passed on from generation to generation and both the plant and its usage traveled with the Europeans to Australia. As claimed by the researchers and the company involved in the discovery, the initial idea goes back to usage in Brisbane, Australia. If hypothetically, this would have been a species brought back by the Europeans from India or what is now Spanish speaking America, this discovery would certainly spark a fierce discussion about the ownership of traditional knowledge. At a pharmacological–clinical level, this discovery highlights the potential to move from one therapeutic field (in this case, topical uses for various forms of skin cancer and precancerous conditions) to other therapeutic uses linked only indirectly with the original use.
Another promising, structurally related, lead is derived from a second Euphorbiaceae, *Homalanthus nutans*, a small rainforest tree used by Samoan healers to treat hepatitis. Its extracts exhibited potent activity in an *in vitro*, tetrazolium-based assay to detect cytopathic effects on HIV-1. It yielded a unique non-tumor-promoting protein kinase C (PKC) activator, prostratin (22), a 12-deoxyphorbol ester, which protects T-lymphoblastoid CEM-SS and C-8166 cells from death due to HIV-1 infection. The compound was first isolated and its structure reported in 1992; thus, this discovery predates that of peplin. Williams et al. demonstrated that prostratin effectively activates HIV gene expression in latently infected Jurkat cells and that it acts by stimulating IKK (IκB kinase)-dependent phosphorylation and degradation of IκBα, leading to the rapid nuclear translocation of NF-κB and activation of the HIV-1 long terminal repeat. Ultimately, prostratin induces the HIV virus to leave cells and thus makes a silent virus accessible to medication. Both ingenol 3-angelate and prostratin rapidly inhibit the HIV virus from infecting cells at an early point in infection. Prostratin has been offered for licensing by the NCI as a candidate anti-AIDS drug, with a significant portion of the potential license income to be returned to the Samoan people.

Betulinic acid, a pentacyclic triterpene found in many higher plants including *Betula* spp. (where it is the most abundant secondary metabolite), was first shown to specifically inhibit the growth of melanoma cell lines. Traditionally, extracts from the *Betula* species have been used topically to treat a variety of inflammatory skin conditions. Species of the genus have been used in North America especially for a variety of gastrointestinal conditions (e.g., removing bile from the intestines, diarrhea, dysentery), as a blood purifier and diuretic, as a general tonic, and as an ointment for persistent scabs and rashes (Cree), gonorrhea (Cree, Iroquois), skin rashes (Algonquin, Cree), and infections (Micmac). Members of the genus are also very widely used in Europe. Historically, uses for dropsy, wounds, and gout were reported, and today it is used popularly to promote hair growth and as a diuretic/cleansing agent. Betula species are currently at the focus of a variety of projects on novel anticancer agents. No direct ethnobotanical link seems to exist between the traditional uses (i.e., as an anticancer agent) and modern biomedical research. This is not surprising, because only few species have recorded uses as anticancer agents. However, many of these uses imply that the extract will modulate the cell cycle, a property that is explored in the development of novel anticancer agents. *Betula* effectively induces apoptosis in neuroectodermal tumors and was shown to be a potent trigger of cell death in human leukemia-derived cell lines. This activity is linked to the activation of NF-κB in a variety of cell lines. Consequently, combination therapies with NF-κB inhibitors would not be of therapeutic benefit, but the drug may have potential if it is used in appropriate combinations. Its potential as an antiviral agent is also under investigation.

The last example is a cure for cancer and tumors from South America. Red Lapacho tea is a canopy tree indigenous to the Amazonian rainforest, which for the first time during the 1960s attracted considerable
attention in Brazil and Argentina. Traditionally, the botanical drug is widely used in local and traditional phytomedicine, usually ingested as a decoction prepared from the inner bark of the tree to treat numerous conditions like bacterial and fungal infections, fever, syphilis, malaria, trypanosomiasis, and stomach and bladder disorders.

As early as 1873, biomedical uses of Red Lapacho (Pau D’Arco) were reported. In 1967, after reports in the Brazilian press, it came to the light of international attention as a ‘wonder drug’. Also in the 1960s, the NCI looked at T. impetiginosa in considerable detail. Two main bioactive components have been isolated from T. impetiginosa: lapachol (23) and β-lapachone (24). β-Lapachone is considered to be the main antitumor compound, and proapoptotic effects were observed in vitro. Some mechanistic studies on this compound’s molecular effects have been conducted. The botanical (drug) material available on international markets today seems to have varying quality and composition, making a specific assessment of the products’ therapeutic claims problematic. Currently, no drug lead based on this species seems to be under development. The bioscientific evidence for products derived from T. impetiginosa is insufficient and highlights both the potential of such new leads and the risks of overstating a (botanical) drug’s therapeutic potential based on limited (generally in vitro) data.75

3.12.2.5.3 Anti-inflammatory natural products

Several compounds are currently under development that may result in clinically approved medications for use in chronic inflammatory conditions. Preparations of Tripterygium wilfordii Hook.f. (Celastraceae) are part of the Chinese traditional herbal traditions (Radix Tripterygi)76 and were first mentioned in the Ben Cao Gang Mu Sbi Yi (1765, Information about Medicinal Drugs: A Monographic Treatment), the classic herbal encyclopedia produced by Li Shizhen (AD 1517–93) during the Ming dynasty. In TCM, it has the functions of dispelling the wind, dehumidification, promoting blood circulation and removing obstruction in channels, subsiding the swelling, relieving pain, killing insects, and detoxifying.77 Preclinical and clinical development has focused on potential uses against cancer, chronic nephritis, hepatitis, systemic lupus erythematosus, ankylosing spondylitis, and a variety of skin conditions.78 In TCM, a patient who has rheumatism would be regarded as having wind, be wet in the body as well as the blood, and her/his Qi being hindered. Dispelling the wind, dehumidification, promoting blood circulation and removing obstruction in channels and reducing the swelling and thus relieving pain are used to treat rheumatism.76,77 Also, in TCM theory, the kidney is in charge of water, that is, is responsible for metabolizing human body water. Therefore, a Chinese doctor would use the functions of promoting blood circulation and removing obstruction in channels as well as inducing diuresis to alleviate edema to cure nephropathy.

Triptolide (25), a deterpenoid epoxide, is essential for the anti-inflammatory and immunosuppressive activities of extracts. As far as one can ascertain, based on uses in TCM, the drug was first further developed in China and then came to the attention of the international research community.

Triptolide inhibited inducible NO synthase (iNOS) gene expression by downregulating NF-κB’s DNA-binding activity and the Jun N-terminal kinase (JNK) or stress-activated protein kinase (SAPK) pathway.79 In other studies,80 the extract of T. wilfordii or triptolide was shown to inhibit lipopolysaccharide (LPS)- and cytokine-induced expression of cyclooxygenase (COX)-2, MMP-3, and MMP-13 in articular chondrocytes, to inhibit the interleukin (IL)-1,- IL-17,-, and tumor necrosis factor-α (TNF-α)-induced expression of the aggrecanase gene in human chondrocytes (triptolide), and to suppress the expression of adhesion molecules E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1).

An exciting example of research driven by traditional knowledge is the discovery of the transient receptor potential vanilloid type 1 protein (TRPV1). These channels were originally cloned while researchers were looking for a molecular target of the pungent compound capsaicin (26) from Mexican hot chili/chilli (spicy varieties of Capsicum annuum L. and C. frutescens L.) and the phorboid resiniferatoxin (RTX, 27) from species of the genus Euphorbia.81 Of course, chilli and paprika have long been used in Meso- and South American cultures, popular as a spice but also as a medicine including for chronic inflammatory conditions. Capsicum annum (which often is less pungent than C. frutescens) originated from Mesoamerica and C. frutescens from the western Amazonian region or Bolivia,82 but today both are part of a universal culture and are generally considered to be an integral part of the medical and culinary traditions on the Indian subcontinent. Chili is a typical Balkan (Hungarian) spice. Multiple medical uses were recorded during the Aztec period, including as a remedy for dental problems, infections of the ear, and various types of wounds as well as digestive problems. Consequently,
chillies were also an important element of tribute requested by the Aztec rulers. During the colonial period, these uses continued and developed further. Now, records of chilli’s use as an aphrodisiac appeared. More recently, \textit{C. frutescens} has been used as a rubefacient to locally stimulate blood circulation. In chemical and pharmacological terms, the development of \textit{Capsicum} spp. is linked to another traditional medicinal plant, \textit{Euphorbia resinifera} Berg (Euphorbiaceae), a large, leafless cactuslike perennial and a native of the Anti-Atlas Mountains of Morocco, which yields euphorbium. Probably, it was King Juba II of Mauretania (50 BC–AD 23) and his physician Euphorbius who discovered the medicinal potential of the resin. Euphorbium has had a medical history of more than 2000 years. This makes RTX one of the most ancient drugs still in use today. Some of its uses, like its application on nerves to suppress chronic pain or on dental cavities to mitigate tooth ache, can be linked directly to the biochemical studies discussed below. The pharmacological interest in this species goes back to the discovery that its key constituent RTX has effects on the transient receptor potential (TRP) channel, similar to capsaicin; this links the history of the drug development of these two botanical drugs.

Both RTX and capsaicin contain a vanilloid (i.e., 3-methoxy-4-hydroxy-benzyl) substructure known to be essential for the potent activity in typical assays of such receptors. The first modern biological studies in the 1950s and 1960s on capsaicin are attributed to the Hungarian pharmacologist Miklos (Nicholas) Jancsó, who died in 1967 and did not see the outcome of his work, which was published by his wife Aurelia Jancsó-Gábor and his pupil Janos Szolcsányi. In 1975 and based on structure–activity relationship studies using capsaicin analogues (capsaicinoids) and fine-tuned dose–response curves in their activities, they first postulated the existence of a specific receptor for capsaicin. Ultimately, these studies transformed the compound from a culinary curiosity to an important pharmacological model and molecular tool for the study of neurogenic inflammation and pain. Empirical evidence for the possibility of desensitization to capsaicin has potential in diverse diseases such as chronic intractable pain, vasomotor rhinitis, or an overactive bladder (Table 2).

Considerable evidence has accumulated bringing attention to the fact that transient receptor potential cation channels (TRPC) function as a molecular integrator not only of the effect of capsaicin but also of a multitude of noxious stimuli including heat, pollutants with negative electric charge, acids, and endogenous proinflammatory substances. The first endovanilloid (i.e., a substance in humans acting like a vanilloid) was the lipid mediator anandamide identified in, 1999, which is also essential as an endogenous cannabinoid receptor ligand. Anandamide is structurally related to capsaicin because both compounds have an amide bond and an aliphatic side chain. Ultimately, these data provide strong evidence for links between the cannabinoid receptor-mediated signaling cascade and TRPC. Thus, the discovery of a receptor for capsaicin has had wide biochemical and pharmacological implications.

Therefore, it is an ideal situation in which to develop anti-inflammatory and nociception-modulation drugs. In 2007, an exciting anesthetic drug lead based on two compounds – a lidocain derivative QX-314 and capsaicin – was developed. Binshtok \textit{et al.} used a combination of these two chemicals to target only pain-sensing neurons, or nocireceptors while leaving other types, such as motor neurons, untouched. QX-314, a charged derivative of
lidocaine, blocks electrical activity in neurons but cannot permeate the cell membranes and induce this anesthetic effect. The excitability of primary sensory nociceptor (pain-sensing) neurons was selectively blocked by introducing the membrane-impermeant compound QX-314 through the pore of the noxious heat-sensitive TRPV1 channel using capsaicin for facilitating selective membrane passage.91 Thus, the active medication would be composed of a pharmacologically active one and one that facilitates this compound’s membrane transport. Is this an ethnopharmacology-driven drug development? Again, it is a complex picture. The concept of a compound targeting the TRPV1 channel is certainly based on the traditional (and very widely distributed) knowledge about chilli’s pungent effects. Detailed molecular understanding of how these ion channels work allowed the development of the strategy to transport the active constituent in a piggyback fashion. Numerous other natural product-derived modulators of these TRP channels are known.86

Two final examples highlight the potential of developing novel anti-inflammatory drug leads using a proinflammatory transcription factor NF-κB as a molecular target. NF-κB is one of the principal inducible transcription factors in mammals and has been shown to play a pivotal role in the mammalian innate immune response and chronic inflammatory conditions such as rheumatoid arthritis. The signaling mechanisms of NF-κB involve an integrated sequence of protein-regulated steps. Many mechanisms are potential key targets for intervention in treating inflammatory conditions. Curcumin is a core compound in turmeric (Curcuma longa, Zingiberaceae) endemic to peninsular India, especially the provinces of Tamil Nadu, West Bengal, and Maharashtra. Turmeric has a small branched rhizome that is bright yellow on the interior. It is used in medicine and widely used in Indian cuisine, for dyeing cloth, and in traditional medicine. In local and traditional medicines, turmeric is considered to be a strong antiseptic and is used to heal wounds, infections, jaundice, urinary diseases, and ulcers and to reduce cholesterol levels. Turmeric, in the form of a paste, has been used to treat external conditions such as psoriasis (anti-inflammatory) and athlete’s foot (antifungal). Therefore, the link with NF-κB signaling is an obvious one, and curcumin has repeatedly shown its inhibitory effects against the signaling cascade of activated NF-κB.92

### Table 2 Capsicum and TRP – an interwoven history

<table>
<thead>
<tr>
<th>Date</th>
<th>Event Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>7000 BC–5000</td>
<td>Archaeological records of Capsicum annuum’s use presumably as a food and medicine in the Teohucán Valley, in Puebla, and in Tamaulipas, México. This includes coprolites and carbonized seeds. These may have been the first cultivated chillis.</td>
</tr>
<tr>
<td>Ca. 2000 BC</td>
<td>Archaeological records of Capsicum frutescens in the graves of Huaca Prieta</td>
</tr>
<tr>
<td>Ca. fifteenth century</td>
<td>Chili (C. frutescens) is used widely in Mesoamerican Indian cultures and is discovered by the Spanish conquistadores. The Aztec term is adopted into Spanish</td>
</tr>
<tr>
<td>1542</td>
<td>Introduction of C. frutescens into India by the Portuguese</td>
</tr>
<tr>
<td>Sixteenth century</td>
<td>Spread of varieties of C. annuum in the eastern Mediterranean, the Near East, and south-central Europe (Hungary)</td>
</tr>
<tr>
<td>1543</td>
<td>Indianischer Pfeffer (C. frutescens) is mentioned in Leonhard Fuchs’ New Kreuterbuch.</td>
</tr>
<tr>
<td>Subsequently, the plant is incorporated into numerous cultures</td>
<td></td>
</tr>
<tr>
<td>1846</td>
<td>Capsaicin is first isolated by L. Thresh</td>
</tr>
<tr>
<td>1850</td>
<td>Turnbull demonstrates that Capsicum extract provides instant relief from toothache, highlighting the therapeutic potential of the species. This line of research is not followed up, however</td>
</tr>
<tr>
<td>Twentieth century</td>
<td>In Europe, C. frutescens (fruit) is used topically for rheumatism</td>
</tr>
<tr>
<td>1919</td>
<td>E. K. Nelson elucidates the structure of capsaicin</td>
</tr>
<tr>
<td>1949</td>
<td>Jancso’ demonstrates that capsaicin produces pain and neurogenic inflammation</td>
</tr>
<tr>
<td>1977</td>
<td>Drosophila TRP channel is identified</td>
</tr>
<tr>
<td>1989</td>
<td>Szallasi and Blumberg demonstrate that RTX from Euphorbia resinifera is an ultrapotent capsaicin analogue</td>
</tr>
<tr>
<td>1990</td>
<td>[3H]-RTX binding sites are described</td>
</tr>
<tr>
<td>1997</td>
<td>Vanilloid receptor 1 (TRPV1) is cloned</td>
</tr>
<tr>
<td>1999</td>
<td>Vanilloid receptorlike channel (TRPV2) is cloned</td>
</tr>
<tr>
<td>2000</td>
<td>TRPV1-deficient mice are developed</td>
</tr>
<tr>
<td>2002</td>
<td>TRPV3 and TRPV4 are cloned</td>
</tr>
<tr>
<td>2002–03</td>
<td>Cold-sensitive TRPs are cloned</td>
</tr>
</tbody>
</table>

Finally, parthenolide from *Tanacetum parthenium*, Asteraceae (feverfew), is a potent inhibitor of NF-κB at low micromolar concentrations. Feverfew has long been used as a bitter tonic and antipyretic. Since the 1990s, some efforts have focused on its use as a potential treatment for migraines. Although parthenolide is not a good drug choice due to its nonspecific cytotoxicity, parthenolide has been studied in great detail from a biochemical–mechanistic perspective. It prevents IκBα and IκBβ degradation and acts against the IKK complex, specifically IKKβ by modifying cysteine 179.93,94 Parthenolide discovery is based on the systematic screening of Mexican Indian medicinal plants used in the context of acute or chronic inflammatory conditions where several sesquiterpene-containing species showed activity.95,96 Parthenolide had not been reported from these species, in fact, but was selected as a model compound for the class. Since that time, numerous members of the sesquiterpene family have been identified as inhibitors of NF-κB.

### 3.12.2.5.4 Antiobesity and antidiabetes drugs

In the 1990s, Fanie R. van Heerden and colleagues at the Council for Scientific and Industrial Research (CSIR) of South Africa isolated two hunger-suppressing pregnane glycosides (28, 29) from *Hoodia gordonii* (Masson) Sweet ex Decne, established their chemical structure, and patented it in 1997.97 Research had already started during the early 1960s focusing on the nutritional value and also any possible long-term toxic effects of food from the veld. The appetite suppressant effect of the plant extracts had already been established in 1983. Without doubt, this discovery was driven by traditional knowledge. *Hoodia pilifera* (L.f.) Plowes (Apocynaceae) and *H. gordonii* are succulent, slow-growing desert plants in southern Africa. Their indigenous names include ghaap, guaap, or ngaap. *H. pilifera* has been known to quench thirst since the nineteenth century, at least.98 The discovery has specifically been linked to the Khoi-San people, but it seems to have been known also in other groups.

Very quickly, this patent aroused the interest of the industry, and a small U.K.-based company (Phytopharm) took a lead further developing it. Key was the extracts’ and compounds’ hunger suppressant and later their antidiabetic effects. In 1998, clinical studies for treating obesity were started and was licensed to Pfizer. The ultimate goal of this R&D effort was a fully licensed medicine on the basis of a characterized extract with a defined amount of the active constituent for the treatment of obesity. Considerable clinical and preclinical research went into developing the drug, but Pfizer unexpectedly returned the license to Phytopharm in July 2003. In late 2004, the food giant Unilever stepped in with the strategic goal to develop a slimming food.99 So far, only limited information about the extracts’ characteristics and their pharmacological effects or clinical effectiveness has been published.100

However, this is the biomedical side. Two other issues are essential, and they highlight the responsibilities of researchers and the industry in ethnopharmacology-driven drug discovery. Because *H. gordonii* is a traditional medicinal and food plant of the San but had been patented without their prior consent, the San of the Kalahari Desert and other stakeholders raised concern about this lack of intellectual and financial recognition. The San and the CSIR finally signed a benefit-sharing agreement in 2004. This was, in fact, one of the first benefit-sharing agreements and gave the San a share of royalties derived from the sale of products containing the patented extract. Specifically, the following agreement was reached:

- CSIR will pay the San 8% of all milestone payments it receives from its licensee, U.K.-based Phytopharm plc
- CSIR will pay the San 6% of all royalties that it receives once the drug is commercially available
- CSIR will make study bursaries and scholarships available to the San community
- CSIR and the San people agree to collaborate in future bioprospecting for the benefit of both parties

This agreement between the San and the CSIR made further development of the product possible. As of today (2008), a second more detailed agreement is due to be signed soon.

The second issue relates to the supply side. As pointed out above, *H. gordonii* are succulent, slow-growing desert plants. The chemical structure of the pregnane glycosides makes synthesis impossible. Also, the commercial goal has been the development of an extract earlier as a medicine and now as a food supplement. With the huge number of obese people in North America, Europe, and other parts of the world, the demand for the botanical drug will be extremely high. Consequently, the commercial production of the plant on farms in the deserts of South Africa and Namibia had to be developed. This has now been achieved, and it is hoped that sufficient material will be available within a few years.
Another now-classical example, the biguanide metformin, which is a semisynthetic derivative of an active natural product, galegine, a guanidine isolated from Galega officinalis L. (Fabaceae, s.str.), is used to treat diabetes. In medieval times, this species was used to relieve intense urination in diabetic people. It also provides an interesting example that although traditional systems of knowledge may lack diagnostic and technical tools to identify certain diseases in a modern biomedical way, such a diagnosis is based on specific signs (or symptoms) a disease produces. Similarly, patients today are diagnosed in one of the primary health care centers and the MDs in these centers normally also prescribe appropriate medication. In many countries like Mexico or India, once a diagnosis is made, patients often go to either local healers or to vendors of herbal and other health care products. From an ethnopharmacological perspective, it is important to understand that diabetes is one at the interface of conventional biomedical and local (or traditional) treatment. Thus, diabetes is for which many of the traditional treatments were, in fact, developed in the last decades by local healers.

The potential of novel antidiabetic medications is enormous. In Mexico alone, for example, a total of 306 species of G. officinalis have been used to treat this disease. Opuntia spp. (cactus pears or prickly pears, Cactaceae) are an essential element of Mesoamerican botanical history. Ripe fruits and nopalcs (or nopalitos, tender cladodes) have been used as food and medicine for centuries. Ill-defined extracts from Opuntia spp. are now widely available over the Internet as a treatment for diabetes and related metabolic disorders for which chemically and pharmacologically characterized extracts are currently under development. Seven other species from México – Cecropia obtusifolia Bertol. (Cecropiaceae), Equisetum myriochaetum Schlecht & Cham (Equisetaceae), Acomusium panamense (Benth.) Yacolev (Fabaceae), Cucurbita ficifolia Bouché (Cucurbitaceae), Agarista mexicana (Hems.) Judd. (Ericaceae), Brickellia veronicaefolia (Kunth) A. Gray (Asteraceae), and Parmentiera aculeata (Kunth) Seem. (Bignoniaceae) – also been studied in detail but have not yet resulted in usable, licensed drugs or nutraceuticals.
3.12.2.5.5 Examples of other drug leads
Numerous examples of new wonder drugs regularly hit the media. It is unlikely that they stand up to such claims, and they regularly highlight the problems associated with poorly defined and characterized starting material. Two examples highlight the core issues.

*Cordyceps sinensis* is a medicinal fungus of TCM. It is a parasite on the larvae of moths (Lepidoptera) of the genera *Hepialus* and *Thitarodes* endemic to alpine habitats (3600–5000 m in elevation) on the Tibetan plateau in southwestern China. In China, *C. sinensis* has a long history of medicinal use. It is thought to have been discovered 2000 years ago with the first formally documented use coming from the *Bencao Congxin* (New Compilation of Materia Medica) in the Qing dynasty in 1757. Overall, little primary ethnomedical data describing the medical uses of *C. sinensis* exist in the literature. Current ethnomedical reports are limited to the use as a general tonic in China and as an aphrodisiac in Nepal. *Cordyceps sinensis* first gained worldwide attention when it was revealed that several Chinese runners who broke world records in 1993 had included this fungus as part of their training program.

Although there are a wide range of reported uses of *Cordyceps* in the literature, the reports that extracts of this fungus may alter apoptotic homeostasis are most intriguing. The reports of clinical trials suggest that *C. sinensis* potentially contains agents that may inhibit apoptosis. These clinical results have stimulated work to assess the ability of *C. sinensis* to inhibit apoptosis *in vitro*, however, the results of these studies are conflicting. The effects may be due to the extracts’ ability to scavenge reactive oxygen species or due to the downregulation of apoptotic genes and the modulation of apoptosis (including downregulation of Fas, Fas ligand, and TNF-α expression) or the induction of apoptosis/cytotoxicity. These conflicting data may be linked to the variability of the strains used and the lack of a consensus strain, variability in the extraction procedures used and/or the need to potentially activate a prodrug present in the extract into an active constituent.

‘Lingzhi’ is the Chinese name of a basidiomycete white rot fungus, *Ganoderma lucidum* (Japanese: Munnertake, Sachitake, and Reishi; Korean: Youngzhi) and related species, which have been used for medicinal purposes for centuries particularly in China, Japan, and Korea. As is often the case with such widely used species, recorded uses vary widely are used to treat migraine, hypertension, arthritis, bronchitis, asthma, anorexia, gastritis, hemorrhoids, diabetes, hypercholesterolemia, nephritis, dysmenorrhea, constipation, lupus erythematosus, hepatitis, and cardiovascular problems. According some researchers, it is used for dizziness, insomnia, palpitations, dyspnea, consumptive cough, and asthma. It is practically impossible to establish how widespread the respective uses have been. Whatever the specific use, the cultural importance of this species has been the driving force for developing potential leads from this taxon. Phytochemical research has focused on bioactive ‘Lingzhi’ polysaccharides and triterpenes, especially ganodermic acid. Extracts from *Ganoderma* have been investigated as potential antitumor and antiviral agents and less so as possible antibacterial agents for antibacterial activity (against Gram-positive bacteria). Some extracts markedly inhibited intracellular signaling and invasive behavior of cancer cells, whereas others were inactive. Also, immunomodulatory effects were observed, which had an impact on various types of cancers. It is too early to assess whether this will result in a successful new drug, but the fact that the extract is the active constituent of this species highlights the need for detailed chemical analysis or metabolomic profiling (cf. Section 3.12.3.3) and for the selection of the most potent extract(s).

3.12.2.6 Ethnopharmacological Information Today
Information on the local and traditional use of plants is scattered in a multitude of sources, and very often such sources are not easily accessible to an international (English-speaking) community because they are written in the national languages of the respective countries.

A well-known and very useful source is a database – NAPRalert, discussed in another chapter of this volume. In addition to many articles in technical journals, there are many monographic treatments available summarizing data for a particular region or country, as well as many ethnobotanical monographs, that can be used as a starting point for research, such as the following:

- Africa incl. the Indian Ocean islands
- South America and North America, including Mexico and the Caribbean

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The best known research facility is the Indian National Institute of Science Communication and Information Resources (NISCAIR) of the CSIR, New Delhi, India.

Ethnobotanical studies are normally conducted with goals that are quite different from the ones in drug development. Therefore, compilations like the foregoing have been used as a starting point in an ethnobotanically driven drug discovery project. However, such information also has a multitude of other uses, as, for example, indigenous groups who want to learn about (often historic) plant use in the cultures and in general the noneconomical benefits of such projects are much higher than the potential but highly uncertain economic gains. The complex and controversial discussion whether such studies should be conducted at all is beyond the scope of this chapter, but its contentiousness will require a continued and open dialogue between all stakeholders. The complex problem has been eloquently highlighted by the late Darrell Posey, an American anthropologist and biologist, who labeled it as the commodification of the Sacred through Intellectual Property Rights.

Ethnobotanical data are generally collected using a series of well-defined methods. Despite these clear standards, many projects suffer from poor botanical documentation or from inadequate anthropological methodologies. Here, we describe general requirements for such projects. In the first instance, an appropriate community or region needs to be selected. All projects can be started only after appropriate permits from relevant national and regional institutions have been obtained (see Section 3.12.2.4). Such projects often last for about 1 year, but there are also examples of shorter projects. In the context of drug development, fieldwork needs to focus on collecting information on the plant’s medicinal use, as well as plants known to be toxic. Essential parts of the process are gathering general ethnographical (background) data, collecting information about how these plants are used, preparing dried herbarium specimens, and collecting samples for further analysis. Complete sets of voucher specimens need to be deposited both in one or more international herbaria that are regionally accessible. Identification generally requires the help of specialists for specific taxa from these institutions, and, of course, the taxonomic validity of the identification needs to be checked using the Index Kewensis (which is at the Royal Botanic Gardens, Kew, U.K.), for example.

Interviews can be conducted either with specialists in local and traditional medicine or with a broader subset of the general population. Specialists can include herbalists, midwives, experts in home remedies (i.e., specialists in treating common illnesses who may not have a specialized status as a healer), bone setters, diviners, and other forms of spiritualist healers. Specialists collect samples known in the region. An important distinction needs to be made between the theoretical and the practical materia medica. The practical knowledge is composed of the prescriptions and plants for which actual evidence for their usage can be collected. The theoretical materia is composed of those preparations that were used historically but that have been replaced by other treatments, by preparations that are known but not used, and, by written documents that list potential local sources of preparations (for details on this distinction and some conceptual discussions, see Lev and Amar). In a more structured interview, the specialists are asked about the uses, preparations, applications of the plants gathered, as well as their concepts about healing. It is essential to transcribe the words in the local language. Information from each healer about the use of one species or preparation for one illness is classed as one use report. For a rapid and simple analysis, these use reports can then be summed up for the various use groups (see below) and taxa. Overall, this results in a set of data that allow a (semi-)quantitative analysis of the data. Many other forms of semiquantification and analysis of the data exist.

In general, this first phase serves to gain an overview of commonly used species and the main concepts of treatment. All this information needs to be stored in appropriate databases. In the case of the abovementioned project, for example, the database consists of 4488 use reports on 614 plant species, contributed by 72 informants. Early on, important decisions about the database’s structure and availability need to be made. For example, it has to be decided who will ultimately be in control of the data that are collected and stored in the database and where it will be held. Will it be in the public domain and possibly available over the Web, or private with limited password-controlled access?
It is beyond the scope of this discussion to provide technical details about which database management system one wants to select. These range from tailor-made ones specific for one project to a simple Access- or Excel-based system. The selection clearly also depends on factors such as the operating system, potential size of database, number of users, and available funds. Currently, relational databases, which use multiple tables of related data, offer one of the best alternatives. The relationships between these tables represent the 'real-world' multidimensions. A surprisingly large problem is the lack of adequate data standards within a single project. This is obviously required for data consistency, exchange of data, and comparative analyses.

In our own work and in order to analyze the cultural importance of the species used and for a cross-cultural comparison, we generally separate the use reports into a series of categories of use, grouping the illnesses into relatively well-defined ethnomedical categories normally based on the human body’s organ system like gastrointestinal, respiratory, and dermatological conditions.

Many criteria exist for selecting possible taxa for further pharmacological and phytochemical analysis. Clearly, already well-studied taxa will often be excluded (dereplication). On the contrary, I have for many years argued that more commonly used species should have priority for further research. The selection may also be driven by preexisting priorities (e.g., specific therapeutic goals of the project).

For further laboratory-based analysis, samples will normally not be processed ‘on site’ and it requires storage of the sample to be used for extraction in an alcoholic solvent or drying of the samples. It is often argued that one should mimic the traditional modes of extraction, but, for example, if the traditional extraction involves fresh plant material, it will be difficult to replicate this in the laboratory if only air-dried material is available. Various extraction solvents have been suggested and used and once more the strategy to be used in a project will depend on its specific requirements and goals of the project. The main general recommendation is to start a dialogue between the scientists involved in the field work and those involved in the pharmacology and phytochemistry well before the collection of the samples starts.

Currently, there is an exciting discussion about which ways to follow on the basis of such information. Many groups follow a systematic in vitro screening approach, which in recent years has become multitarget (many such studies have been published, e.g., in the Journal of Ethnopharmacology). However, few of these extracts or compounds are then taken further.

An alternative approach has been proposed by Graz et al. and by Raza. The latter argues for a role of physicians at all stages of the drug development process from the initial fieldwork (where she/he interprets traditional terminologies using biomedical modern counterparts, identifies the disease for which a local and traditional remedy is used, and examines patients consuming herbal remedies) to clinical studies on herbs as well as the study of their potential interaction with modern medicines. Graz et al. suggest designing clinical studies appropriate for traditional medicines and for use in the field. Core methods are the retrospective assessment of treatment outcome and population surveys, the prognosis–outcome method (with modern physicians observing progress of patients treated by a traditional healer), or the dose-escalating prospective study (detecting a dose–response phenomenon in humans). In each case, clinical data are generated at an early stage and allow a much more detailed understanding of the local and traditional medicines used as well as of the treatments and their outcome in general. Arguably, such strategies will work best for diseases prevalent in the regions of study (e.g., infectious diseases) and thus may not be as useful, for example, for those diseases that are currently at the center of most commercial drug development programs.

This short overview cannot be a comprehensive review of the relevant methods, but offers some general strategic hints highlighting the complexity and multidisciplinarity of such projects.

### 3.12.3 Today's Core Challenges

#### 3.12.3.1 The Stakeholders

Until the implementation of the CBD (cf. Section 3.12.2.4), the main stakeholders were scientists (generally in large scientific research institutions like the US NCI, the pharmaceutical industry, and some university-based researchers), medical doctors, and their legal representatives.

With the changes in the legal framework, indigenous groups in the ‘provider countries’, NGOs and, most importantly, the provider countries themselves entered the scene. Few of these groups had or have an
understanding of the process of drug discovery and its duration, but they are united by an interest in protecting
the rights of those who represent the providers. Clearly, there is a need for a dialogue between all groups
involved.

The example of galanthamine (Section 3.12.2.3) points to another core challenge. Drug development has
always been a lengthy process and the initial development of this drug started in the Soviet Union shortly after
World War II. When the compound became of interest for treating Alzheimer’s disease 40 years later, the
Soviet Union had disappeared and, consequently, one has to ask whether it will be possible to develop a system
that could withstand such political changes.

3.12.3.2 Neglected People and Diseases

There can be no doubt that diseases for which no industrial R&D activities exist remain a truly neglected area
of medical science and practice. There is no standard global definition of neglected diseases. ‘Neglect’ has
become one of the most commonly used words to describe certain diseases primarily, if not exclusively,
affecting poor populations in developing countries. The key elements are diseases affecting principally poor
people in poor countries, for which health interventions – and R&D – are seen as inadequate. Ten neglected
(‘tropical’) diseases have been listed by the World Health Organization Special Programme for Research and
Training in Tropical Diseases (WHO/TDR). These are leishmaniasis, schistosomiasis, onchocerciasis, lymph-
atic filariasis, Chagas disease, malaria, leprosy, African trypanosomiasis, tuberculosis (TB), and dengue.
Other diseases commonly considered to be neglected include hookworm, roundworm, or diarrheal illnesses,
Buruli ulcer, congenital syphilis, and trachoma. Despite various bacterial threats, such as multiply drug-
resistant strains, and emerging pathogens like mycoplasma, most large pharmaceutical companies have
abandoned antibacterial drug discovery. Bacterial and mycoplasmatic diseases are therefore also considered
to be neglected.148,149

As pointed out in a joint policy document by the London School of Economics and Political Sciences and the
Wellcome Trust,150 in the context of neglected diseases the (commercial) Intellectual Property (IP)-driven
innovation model has some limitations. There is no public control over industry’s R&D agenda, which (being
commercially driven) may not coincide with the areas of greatest public health need. Limited public control
over the pricing of the final product, when this occurs, can also result in reduced patient access if purchase funds
are tight since fewer daily doses can be purchased at the higher monopoly price than at the lower competitive
price.150 Natural products offer much more realistic opportunities for developing such low-cost innovative
drugs. The classical example of a drug used against neglected diseases is *A. annua* and the sesquiterpene lactone
qinghaosu derived from it (see above). The advantages of drug development projects based on plants
traditionally used in the treatment of these conditions are the direct link between the traditional use, the
drug development project, and hopefully the opportunity to develop these products at lower costs. Lastly, some
of these products, if proven to be safe and efficacious, may be used as phytomedicines produced locally.

3.12.3.3 Extracts as Medicines?

In recent years, novel opportunities have been subsumed under the idea of the ’omics revolution. Metabolomics, for example, ideally will qualitatively and quantitatively analyze all metabolites in an organism (e.g., a medicinal plant) or a complex drug. As pointed out by Verpoorte et al.,151,152 this is a very ambitious goal, and it is questionable whether this is a realistic goal. This approach allows a systematic investigation of complex mixtures and specifically to link phytochemical analysis with other strategies (such as *in vitro* or *in vivo*
screening for biological activity or toxicity, morphological plant diversity, and ecological parameters).
Specifically, as it relates to the study of medicinal and food plants, the main challenge is to understand the
complex effects of such extracts. This may offer unique and novel opportunities to develop new medicines
based on local and traditional knowledge, but the true potential of such an approach remains to be seen.

Our group investigated two poorly studied traditional preparations of cannabis (*Cannabis sativa* L.,
Cannabidaceae, various cultivars), the water extracts and tinctures, in order to evaluate the overall
metabolite profiles and the relative amount of $\Delta^9$-tetrahydrocannabinol (THC) with respect to $\Delta^9$-THC-
acid and other cannabis constituents using a combination of NMR analysis (diffusion-edited $^1$H NMR
(1D DOSY) and ^1^H NMR with suppression of the ethanol and water signals) and in vitro cell assays (inhibition of NF-κB activation). Depending on the extraction procedure, the extracts were highly variable with respect to constituents including δ⁹-THC and δ⁸-THC-acid. With this method, it was possible, without any evaporation or separation step, to distinguish between tinctures from different cannabis cultivars. This case highlights the potential of optimizing an extract based on the effects of a specific target (or potentially a series of targets).¹⁵³,¹⁵⁴ Here it serves as an example of developing extracts into medicines (see also Section 3.12.2.3) and the specific case of cannabis is discussed in much greater detail in another chapter of this volume.¹⁵⁵ In another example, Boelsma et al.¹⁵⁶ investigated the effect of G. biloba extract EGb 761 on skin blood flow in healthy volunteers using laser Doppler flowmetry and the accompanying changes in urinary metabolites in urine using a combination of NMR spectroscopy and multivariate data analysis (MVDA). Following EGb 761 treatment, the overall mean skin blood flow was significantly reduced as compared with placebo. NMR/MDVA analyses showed that urinary metabolic patterns differed depending on the change in baseline blood flow after treatment. The results highlight the usefulness of metabolic fingerprinting as a tool for understanding biochemical changes and associated functional changes and, therefore, have implications for drug development.

3.12.3.4 Let Food Be Your Medicine and Let Medicine Be Your Food

As it becomes obvious from the above, and as pointed out by others, the borderline between food and medicine is blurred.¹⁵⁷–¹⁵⁹ Similarly, anthropologists¹⁶⁰–¹⁶² have argued that there exist strong links between food and medicines in indigenous societies. Today, we are very conscious about this, and this chapter highlights that the decision whether an ethnopharmacology-driven research project has a new food supplement or a new medical product as its ultimate goal is often arbitrary. The case of Hoodia demonstrates this very clearly. In legal terms, in many countries a product is considered to be a medicine if it makes specific claims for treating or preventing a certain illness and a health food if it has general health beneficial effects as well as alleviating a specific illness or syndrome. Consequently, ethnopharmacology-driven drug development has a broader scope for applications than approaches based on medicinal chemistry, for example. Arguably, especially in the case of Europe (and presumably also North America and Australia/New Zealand), from an industrial perspective, the greatest opportunities lie in developing novel food supplements/health foods/traditional herbal medical products or ‘cosmeceuticals’ based on local and traditional knowledge.

3.12.4 Conclusion: People, Plants, and the Future of Medicines

This chapter reviewed some of the many medicines and drug substances that are based on local and traditional knowledge. Such a review needs to be examplatory and selective. Overall, it highlights that oral and written local/traditional knowledge has provided many unique novel leads and that such an ethnopharmacological approach continues to be a fascinating and particularly valuable strategy. As we pointed out recently, the world’s societies are in a continuous process of globalizing selected elements of local knowledge¹⁵⁷ and equitable benefit sharing as well as the development of mechanisms to safeguard such knowledge for future generations¹⁶³ in the regions where this knowledge developed will have to be an essential element of any R&D strategy.

Ethnopharmacology and drug development can be understood only if a truly multidisciplinary approach is taken and this is one of the most exciting and promising challenges of the field – it requires a dialogue not only between disciplines but also between cultures. Ethnopharmacology-driven drug development uses a unique knowledge-based strategy, which will hopefully result in many more new medicines for use by all humans. The needs of those who require such new and better medications most and who can least afford them have to come at the forefront of decision makers in industry and politics. Locally and traditionally (mostly plant based) used medicines offer unique opportunities provided that there exists the willingness to support such research, which generally is at the border between basic and applied research.
Abbreviations

CBD Convention on Biological Diversity (1992) also known as Rio Convention
COX cyclooxygenase
ICAM 1 intercellular adhesion molecule 1
IKK IκB kinase
IL interleukin
iNOS inducible NO synthase
IP Intellectual Property
JNK Jun N-terminal Kinase or Stress Activated Protein Kinase
LPS lipopolysaccharide
MMP (3/13) Matrix metallopeptidase (3/13)
NCI National Cancer Institute
NF-κB nuclear factor kappaB
PKC protein kinase C
TCM traditional Chinese medicine
THC tetrahydrocannabinol
TNF-α tumor necrosis factor α
TRIPS Trade-Related Aspects of Intellectual Property Rights
TRPC transient receptor potential cation channels
TRPV transient receptor potential vanilloid type [1–4] protein
VCAM-1 vascular cell adhesion molecule-1
WTO World Trade Organization

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