Can an Apple a Day Keep the Doctor Away?

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Abstract: The modern pharmaceutical industry based on synthetic chemistry severed the historical connection between plants, food and medicines. The growing costs of discovering new chemical entity-based drugs through high throughput screening methods may yet again reconnect plants and human health at a new level of technological sophistication. Multi-component botanical therapeutics that comprise functional foods, dietary supplements and botanical drugs hold several advantages over conventional drugs that may earn them a more prominent place in the medicine of the future. They can deliver mixtures of multi-functional molecules with potentiating and synergistic effects and pleiotropic targeting at a reasonable cost and with fewer regulatory constraints. They are well suited for long-term disease prevention in an era of genetic testing and increased life expectancy. They also provide additional vehicles for delivering health and wellness. Technologies that address the needs of discovery, development and manufacturing of multi-component botanical therapeutics are emerging. They include computational and bioinformatics approaches, cell based gene expression and high-content screening systems, and phytochemical elicitation and unique plant cultivation / extraction methods designed to optimize the production of bioactives, standardize overall extract composition and assure batch-to-batch product consistency. Nevertheless, multi-component botanical therapeutics carry risks associated with potential interactions with conventional drugs and adverse reactions, which are difficult to detect and diagnose. They face problems of acceptance by the medical community and pharmaceutical industry, safety and efficacy validation, poor standardization and quality control, and difficulties in identifying active ingredients and determining their complex mode(s) of action. Solving these problems will accelerate the merger of grocery stores with pharmacies and agriculture with chemical manufacturing and provide physicians and patients with broader and more individualized choices for disease prevention and treatment.

Key Words: phytochemicals, botanicals, botanical therapeutics, botanical drugs, nutraceuticals, functional foods, dietary supplements, drug discovery, natural products, human health.

SINGLE INGREDIENT DRUGS *VS*. BOTANICAL THERAPEUTICS

Throughout human history most medicines were derived from natural products, often from plants, and delivered as foods or extracts and powders (see Inset). Synthetic chemistry broke the connection between plants and human health, making Western medicine primarily dependent on pharmaceuticals based on single synthetic or naturally-derived molecules delivered orally, topically or injected. This approach revolutionized medical care in the 20th century and provided powerful new tools to cure diseases, reduce their symptoms and extend human life. The race to develop new chemical entities (NCEs) as components of proprietary drugs led to a revolution in synthetic chemistry and to the development of combinatorial, computational and high throughput approaches to drug discovery. While a number of drugs are still isolated from the natural source or prepared by semi-synthesis from a natural precursor (Table **1**), the pharmaceutical industry is becoming less interested in plants as sources of new drugs [1]. Researchers who are still using phytochemicals in their drug discovery programs view them as initial leads to be improved through structure activity relationship (SAR) programs and valuable only if a cost effective chemical synthesis route to manufacturing can be established. The most commonly cited reasons for the diminished interest in phytochemicals as sources of NCEs are:

1. **Incompatibility of a High Throughput Format with Complex Botanical Extracts.** Polyphenols, pigments, saponins and other constitutive components of plant extracts often interfere with *in vitro* protein binding and enzyme activity assays, generating a high number of false positives. As a result, some groups involved in plant-based drug discovery fractionate plant extracts before primary screening. This adds significant cost to the discovery process, while increasing the chances of discovering new leads.
2. **Reproducibility**. Plants change their biochemical composition depending on the environmental conditions and harvest time. Biotic and abiotic stresses encountered at the time of harvest may dramatically change the chemical composition of a plant extract and its pharmacological activity [2]. Thus, the same plant species harvested at different times and from different locales may not yield reproducible screening results. These differences may even be expressed during different times of the day, since the transcription of at least some genes involved in plant secondary metabolism, i.e. flavonoids, show diurnal circadian fluctuations [3].
3. **Price.** High throughput screening technologies require thousands of samples every week. It is prohibitively laborious and expensive to produce enough unique natural product-based extracts to satisfy the voracious

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1381-6128/04 $45.00+.00

Inset

SHORT HISTORY OF BOTANICAL THERAPEUTICS

- Before 3000 B.C. Ice-man is frozen carrying medicinal herbs
- Around 3000 B.C. First Sumerian writings mention medicinal plants
- Around 2700 B.C. Shen Nung, Chinese emperor, publishes written records of medicinal plants and their uses called *Pen T-Sao*
- 1700 B.C. *Law Code of Hammurabi* from Mesopotamia mentions many medicinal plants
- 1500 B.C. Eber's papyrus of herbal remedies from Egypt containing hundreds of herbal prescriptions (possibly copied from the earlier version)
- 1000 B.C. Indian Ayurvedic herbal remedies recorded
- 340 B.C. Greek Theophrastus writes two books on uses and cultivation of medicinal plants
- 100 B.C. Krateus, a Greek herbalist, publishes the first illustrated book on medicinal plants
- 60 A.D. Pliny writes *Natural History*, that contains medicinal and other descriptions for over 1000 plant species
- 78 A.D. Greek Dioscorides records the collection and use of herbal drugs in *De Materia Medica*, which becomes a classic text
- Around 200 A.D. Chang Chung-Ching writes books that become basics of Chinese and Oriental herbal medicines.
- 800 A.D. Arabs establish private drug stores selling many herbal remedies
- Around 1000 A.D. Ibn Sina (Avicenna), born in today's Uzbekistan, writes several texts in Arabic on healing and herbal medicines that remain classics for centuries
- Around 1240 A.D. Ibn al-Baytar, the greatest herbalist of medieval Spain, describes more than 1000 botanical medicines
- 500-1200 A.D. European monasteries maintain medicinal gardens and preserve knowledge of herbal medicines
- 1565 A.D. Pietro A. Mattioli publishes the second and most comprehensive addition of his historically acclaimed Commentary on Dioscorides
- 1640 A.D. Spanish bring back *Cinchona* bark from new world for malaria treatment
- 1785 A.D. William Withering, working in England, discovers the use of foxglove, *Digitalis purpurea*, to treat heart diseases
- 1795 A.D. British Navy issues lemon juice to sailors to prevent scurvy, based on earlier studies of John Woodall and James Lind –functional foods are born
- 1803 A.D. Wilhelm Serturner, working in Germany, isolates morphine and other alkaloids
- 1820 A.D. P-J. Pelletier and J.-B.Caventou, working in France, isolate quinine, emetine, strychnine and brucine
- 1838 A.D. Raffaele Piria, working in Italy, isolates salicylic acid from willow bark
- 1897 A.D. Bayer, a German company, synthesizes Aspirin and modern pharmaceutical industry is born.
- 1971 A.D. US National cancer institute discovers Taxol® from *Taxus brevifolia*
- 1978 A.D. Germany establishes Commission E monographs for herbal medicines
- 1984 A.D. National Cancer Institute (NCI) endorsed messages about the benefits of dietary fiber
- 1988 A.D. K. During and A. Hiatt independently produce human anti-bodies in tobacco
- 1992 A.D. Convention on Biological Diversity (CBD), signed at the UN Earth Summit in Rio de Janeiro, establishing national ownership of plants and biochemical diversity
- 1994 A.D. Dietary Supplement Health and Education Act (DSHEA) is signed into law. US nutraceutical industry gains legitimacy and expands rapidly.
- 1997 A.D. US FDA approves the first food specific health claim that soluble fiber may reduce the risk of heart disease
- Around 2000 A.D. Merck and Co., Inc. and other pharmaceutical companies dramatically downsize their phytochemical-based drug discovery
- 2000 A.D. US FDA issues Botanical Drug guidance
- 2001 A.D. US FDA allows sales of galanthamine for Alzheimer's disease - the latest approved plant-derived drug
- 2003 A.D. US FDA issues Guidance for Qualified Health Claims in the Labeling of Conventional Human Food and Human Dietary Supplements

Table 1. Pharmaceuticals or their Precursors Derived from Plants. Partially Adapted from [7, 86]

Alkaloids	**Terpenes and steroids**
Atropine, hyoscyamine, scopolamine	Artemisinin
Camptothecin[a]	Diosgenin[a], hecogenin[a], stigmasterol[a]
Cocaine	Taxol and other taxoids[a]
Codeine, morphine	Digoxin, digitoxin
Colchicine	**Phenolics**
Emetine	Podophyllotoxin[a] – lignan (Etoposide)
Galanthamine	Sennosides A and B – hydroxy-anthracene glycosides
Nicotine	**Others & Mixtures**
Physostigmine	Ipecac
Pilocarpine	
Reserpine	
Tubocurarine	
Quinidine	
Quinine	
Vinblastine, vincristine	
Yohimbine	

[a] Most often used as precursors in chemical synthesis of final products.

sample appetite characteristic of modern screening technologies that often emphasize quantity and not quality of samples.

4. **Difficulty in isolating an active ingredient.** Today's high throughput NCE discovery format requires that active ingredients are characterized rapidly and that the activities associated with previously characterized compounds are ignored. This is often difficult to achieve in the time frame of the screen. Since the extract's activity may be the result of potentiating effects of several compounds, it may be lost or reduced during the isolation process.

5. **Long resupply time.** Isolation and characterization of actives require gram quantities of the extract that is usually not available at the time of screening. Often resupply must come from exotic plant sources and remote geographical locations. Since fungal and bacterial cultures can be scaled up from the original supply, some natural product-based drug discovery programs may favor microbial sources over plants. Multicellular marine organisms probably constitute the least 're-suppliable' sample sources, unless the lead is produced by the symbiotic microbes commonly associated with these organisms.

6. **Geopolitical reasons.** The Convention on Biological Diversity (CBD) adopted by the UN Earth Summit in Rio de Janeiro and signed by over 170 countries, asserts that countries own their biodiversity and, thus, requires that benefit sharing negotiations take place before local biota can be sampled. Resources and time necessary to complete these legal arrangements and the intrinsic costs of benefit sharing make collections in many regions difficult or impossible [4].

Despite major technological advancements in genomic research, screening, computational design and combinatorial chemistry, it is becoming progressively more difficult and expensive to discover and develop NCEs that are more efficient than those already on the market [5]. That raises the possibility that the synthetic NCE paradigm, so successful for the last century and still the mainstream of the pharmaceutical industry, is slowly coming to an end. Remarkably, the number of NCE drugs developed each year based on natural products remained relatively constant over the last 22 years even while the interest in these sources decreased. In the areas of cancer and infectious diseases over 60% and 75% of new drugs, respectively, were of natural origin or were synthetics modeled on natural lead compounds, with other clinical areas being not far behind [6].

MULTI-COMPONENT BOTANICAL THERAPEUTICS: FOODS OR DRUGS

Can plants still contribute to whatever the future of medicine may be? The answer may be positive, but that contribution may come not in the form of new skeletons for synthetic NCEs, but in more esoteric and not yet fully validated categories that include functional (medicinal) foods, botanical dietary supplements (nutraceuticals) and botanical drugs. These plant-based agents belong to a newly defined category of multi-component botanical therapeutics (Table **2**) that are being developed in academic and industrial laboratories throughout the world [7]. Other botanical therapeutics include single-component drugs originally derived from plants, such as taxol or atropine, and the drug-like recombinant proteins manufactured in plants, such as human anti-bodies (Table **2**).

The conceptual differences between functional foods [8] and botanical drugs, or their poorly regulated, validated and standardized cousins, botanical dietary supplements, is that the latter two groups may supply a more concentrated, higher dose of pharmacologically active compounds. This dose can be strictly controlled and administered in the form of conventional medicines, i.e. tablets or capsules. In addition, botanical drugs and botanical dietary supplements may be derived from a broader variety of plants than normally present in the human diet. Botanical drugs, functional foods

Table 2. Categories of Botanical Therapeutics

Category	Description	Example
Multi-Component Botanical Therapeutics		
Botanical Drugs	Clinically validated and standardized phytochemical mixtures developed through the FDA	None in the U.S., several in clinical trials
Dietary supplements/ Nutraceuticals	A plant component with health benefits developed under DSHEA and carrying only structure-activity claims	Garlic, St. John's Wort or Echinacea extract
Functional/Medicinal foods	A plant-derived food engineered or supplemented to provide health benefits	Modified canola oil, high fiber cereals, golden rice
Single-Component Botanical Therapeutics		
Drugs (NCE)	Single active ingredient, NCE-based pharmaceuticals originating from plants developed through the FDA	Vinblastine, Taxol or Aspirin
Recombinant proteins	Pharmaceutical protein expressed and isolated from plants	Human anti-bodies

and dietary supplements have recently received a boost by the US Federal Drug Administration (FDA) in the form of Botanical Drug Guidance (http://www.fda.gov/cder/guidance/1221dft.htm) and Qualified Health Claims in the Labeling of Conventional Human Food and Human Dietary Supplements (http://www.cfsan.fda.gov/~dms/hclmgui3.html). The FDA proposed that botanical drugs, representing standardized and partially characterized multi-functional and multi-component plant extracts with a safe history of human use may be developed through abbreviated pre-clinical and clinical protocols. No botanical drugs are currently sold in the US with the possible exception of syrup of ipecac and digitalis extract that were accepted by the US pharmacopoeia long before the introduction of the botanical drug category. Qualified health claims will provide the food and nutraceutical industry with relatively well-defined criteria for claiming health benefits for their products based on scientific evidence. The new labeling system proposed by the FDA will use a letter-grading system for the strength of scientific evidence behind the claim, with an "A" for those with significant validation, down to a "D" for the weakest. Currently allowed health claims that relate to plant-derived foods sold in the US are summarized in Table **3**. Botanical dietary supplements currently sold in the US are defined by the Dietary Supplement and Health Education Act of 1994 (DSHEA). They also belong to the multi-component botanical therapeutics but are less regulated, standardized, validated and often controversial versions of botanical drugs. In exchange for the absence of disease prevention and treatment claims, DSHEA does not require proof that supplements are safe and effective. Finally, single component botanical therapeutics [7] are basically identical to the conventional drugs of botanical origin, and thus are not the focus of this review.

In contrast to the US, where most multi-component botanical therapeutics are marketed under DSHEA, the majority of the developed countries define them as drugs and/or regulate them more rigorously. European regulations are currently in a state of flux and vary between different countries. However, most of the multi-component plant extracts, marketed as dietary supplements in the US, are sold as drugs in Europe and undergo a more rigorous regulatory review. This practice may change when the European Union finalizes four sets of regulations currently under development: Nutrition Health Claims Directive, Fortified Foods Directive, Sports Nutrition Directive, and Traditional Herbal Medicinal Products Directive. In Japan, which according to World Health Organization has the highest per capita consumption of herbal medicines in the world, physicians still widely prescribe Kampo herbal medicines (botanical drugs) that are regulated and certified by the Japan's Ministry of Health and Welfare and the Japan Kampo Medicine Manufacturers Association. Kampo medicines sold by Japanese doctors and drug stores are generally covered by health insurance. Japan also has a category of Food for Specified Health Uses (FOSHU), which requires establishing safety and efficacy with animal and/or human studies, publication of data, identification of key component(s), physio-chemical characterization, determination of the appropriate dose levels and approval of the product label.

Multi-component botanical therapeutics may contribute to the future of conventional medicines because of several advantages over conventional pharmaceuticals: They may deliver a complex combination of interacting compounds with pleiotropic effects and, in the case of the functional foods, in doses that exceed those of conventional oral pharmaceuticals. They may also be cheaper and faster to develop and manufacture, resulting in lower costing medicines with a larger emphasis on preventative care. These advantages are further analyzed below.

INTERACTIVE NATURE OF PHYTOCHEMICALS

In contrast to higher animals, plants synthesize bewildering arrays of organic molecules with functions that have puzzled generations of phytochemists. Our survey of different databases and publications indicates that at least 200, 000 phytochemicals, excluding DNA-encoded proteins and peptides, have been characterized, still representing a small fraction of phytochemicals produced by the 250, 000

Table 3. Allowed Health Claims for Plant-Derived Foods Sold in the US. (Adapted from http://www.cfsan.fda.gov/~dms/lab-ssa.html and http://www.cfsan.fda.gov/~dms/qhc-sum.html)

Health claims authorized before July 2003*
Fiber (fruits, vegetables, grains) may reduce cancer risk
Soluble fiber (fruits, vegetables, grains) may reduce coronary heart disease risk
Soluble fiber (whole oats, psyllium seed husk) may reduce coronary heart disease risk
Fruits and vegetables may reduce cancer risk
Soy protein may reduce coronary heart disease risk
Plant sterol/stanol esters may reduce risk of coronary heart disease
Adequate amount of folate (Fruits, vegetables, grains) may reduce neural tube birth defects
Qualified health claims authorized after July 2003**
Antioxidant vitamins (E and C) may reduce the risk of certain forms of cancer
Nuts may reduce the risk of heart disease
Walnuts may reduce the risk of heart disease
Soy-derived phosphatidylserine may reduce the risk of dementia in the elderly

*Based on NLEA, Nutrition, Labeling and Education Act of 1990 and FDAMA, FDA Modernization Act o f 1997.

**Based on Interim Procedures for Qualified Health Claims in the Labeling of Conventional Human Food and Human Dietary Supplements (http://www.cfsan.fda.gov/~dms/hclmgui3.html).

plant species growing on earth. In 1998, the number of known phytochemical structures was closer to 140, 000 [9]. Major improvements in analytical technology have increased the rate of phytochemical identification every year, but it is widely believed that the great majority of phytochemicals have not been characterized or functionally tested [7, 10]. While some of these compounds, often called secondary metabolites, play defense, communication and internal signaling functions or are biochemical intermediates or catabolites, the functions of many others remain to be determined. It is becoming clear that these compounds may exert their bioactivities by interacting with other molecules, rather than acting alone as probably most of the compounds produced by prokaryotes do. It is possible that the evolutionary significance of a large number of phytochemicals present in each plant lies in their complex mutually potentiating effects that provide protection against diverse pathogenic microbes and herbivores and help to assure more reliable signaling to pollinators and other beneficial organisms. Modern medicine has only recently learned how rapidly pathogens and cancer cells can develop resistance to single ingredient drugs, necessitating the administration of complex drug cocktails to circumvent or delay the development of resistance. Plants may have learned this strategy very early in their evolution.

For example, *Berberis fremontii* produces both antimicrobial berberine alkaloids and inhibitors of a bacterial multidrug-resistant pump that strongly potentiate the antibacterial activity of berberines [11]. *Coptidis rhizoma*, a medicinal herb with an anti-cancer effect, also contains berberine as major bioactive principle. A recent study showed that the extract of this plant has a more potent antitumor activity than pure berberine and that the effects of the extract and pure berberine on the anti-cancer genes do not fully overlap [12]. The root extract of a *Tripterygium wilfordii*, used as a traditional Chinese medicine, has a strong anti-inflammatory effect based on blocking the expression of a number of pro-inflammatory genes including those encoding cyclooxygenase-2, inducible nitric oxide synthase and several inflammatory interleukins. A recent phase I/II double-blind, placebo-controlled trial in the US has confirmed that effect [13, 14]. Although the main active ingredient of this extract, diterpenoid triptolide, has been identified, it is too toxic and less effective unless given as a part of the root extract, suggesting that other unidentified extract components increase its safety and, possibly, efficacy [15]. Similarly, mixtures of plant flavonoids had a synergistic effect on anti-fungal activity greater than the sum of the effects of their purified components [16]. Plants that have mild diuretic properties, such as *Solidago virgaurea* (goldenrod), *Betula* (birch), and *Ononis spinosa* (rest harrow), contain flavonoids, saponins, and volatile oils that in combination are responsible for the overall diuretic effect that can not be reproduced by a single component [17]. Potentiating phytochemical interactions between the terpene lactones (ginkgolides and bilobalide) that are platelet-activating factors and the flavonoid antioxidants (mostly proanthocyanidins and rutins) and microcirculation enhancers may account for wide range of effects of *Ginkgo biloba* extract [18]. While lycopene in tomatoes has been associated with decreased risk of cancer and cardiovascular diseases in numerous studies [19-21], whole tomato powder reduced carcinogenesis in rats more than pure lycopene [22].

Negative interferences occur when certain components of the mixture inhibit full biological activity of other components. For example, although tea has a higher caffeine

content than coffee, its stimulating activity is less marked because of the interaction of caffeine and proanthocyanidins in freshly brewed tea that limit its bioavailability [23]. Similarly, citrus pectins significantly depressed the bioavailability of beta-carotene [24].

Also, the effects of drugs can be altered by commonly consumed phytochemicals. For example, furanocoumarins and to a lesser extent flavonoids present in grapefruit juice substantially alter the pharmacokinetics of calcium blockers and other drugs by suppression of the cytochrome P 450 enzyme CYP3A4 in the small intestine wall, in essence increasing their effective dose. Grapefruit juice may also inhibit intestinal P-glycoprotein-mediated efflux transport of drugs such as cyclosporine to increase its oral bioavailability [25, 26]. Recently, phytochemicals contained in many botanical dietary substances were shown to modulate effects of many conventional drugs, raising the concerns of doctors and regulators [27, 28]. Some of this attention was focused on the interactions of St John's Wort-derived nutraceuticals with a variety of drugs such as cyclosporin, indinavir, warfarin and psychotropic and narcotic agents [29, 30].

Successful treatment of complex chronic diseases almost always requires multi-component therapy to deal with multiple symptoms and causes. Since current regulations make the development of multi-component pharmaceuticals impractical and expensive, the common solution to treatment is to provide patients with a cocktail of drugs, most with a single active ingredient. The realities of an intensely competitive and regulated pharmaceutical industry dictate that more efforts are placed on the study of negative drug-drug interactions than on the evaluation of potential synergy between existing drugs or drugs and foods. Multi-component botanical therapeutics may become particularly valuable in the long-term prevention and treatment of complex diseases requiring extended administration and pleiotropicaction.

So why did plants evolve compounds that effectively interact with therapeutic targets in humans? While antimicrobial and selectively cytotoxic compounds protect plants against infectious diseases and herbivory respectively, we can safely assume that most of the other pharmacological activities of phytochemicals are coincidental. Yet, most have evolved to play some function in biological systems and that should make them better therapeutic agents than randomly chosen synthetic chemicals.

EMPHASIS ON PREVENTION

Sequencing of the human genome has brought us closer to understanding the genetic basis of human diseases. The ever increasing rate of gene discovery and their mutations predisposing people to diseases constantly improves the understanding of the long-term risk factors faced by carriers of these genes and their children [31, 32]. For example, easily testable mutations in the tumor suppressor genes *BRCA1* and *BRCA2* result in a dramatic increase in the risk of breast and ovarian cancer [33]. In families with multiple cancer cases, the estimated lifetime risk of breast cancer is >80%, and the lifetime risk of ovarian cancer is 40 to 65% for *BRCA1* carriers and 20% for *BRCA2* carriers [34]. Mutations responsible for increased risks of other cancers, diabetes, cystic fibrosis, obesity, autoimmune, psychiatric and neurological diseases are being rapidly catalogued and genetic tests for these mutations developed and transferred to the clinic. Furthermore, new technologies allow the creation of individual disease risk profiles based on single nucleotide polymorphism (SNP) mapping, gene-expression profiling or proteomics [35, 36].

The advent of genetic testing is impaired by the failure of modern medicine to effectively respond to identified health risks, since treatment and not prevention is still at the core of the health industry. Yet it creates a powerful force for the development of a new generation of preventative therapeutics. Botanical therapeutics and functional foods in particular, may serve as the first line of defense against genetic risk factors, because of their innate emphases on prevention. For example, about 35 cancer preventive plant-food sources have been identified by the National Cancer Institute (NCI) including garlic, cruciferous vegetables, ginger, onion, tomatoes and legumes [37, 38]. Many phytochemicals associated with foods such as allicin, polyphenols, isoflavones, anthocyanins, omega-3 fatty acids and fiber are implicated in the prevention of cardiovascular diseases [39-42]. Functional food claims approved by FDA (Table **3**) represent some of the best-tested examples of foods that can help with disease prevention. However, food companies are often reluctant to expend resources on obtaining qualified health claims on foods because of the inherently generic nature of this industry and the questionable success of the currently marketed functional foods. On the other hand, doctors are also somewhat reluctant to recommend a functional food because of the limited training they receive in this area. Nevertheless, the accumulation of solid scientific evidence on the benefits of functional foods and some dietary supplements should reverse the slow rate of their acceptance by the medical community.

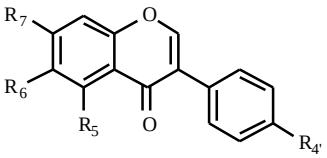
COMBINATORIAL PHYTOCHEMISTRY

As mentioned above, the full health benefits of multi-component botanical therapeutics can rarely be reduced to a single pharmacologically active ingredient. Most foods or botanical extracts contain phytochemicals belonging to several groups of health promoting compounds that likely interact with each other. In addition, any natural product isolated from a plant is usually a member of a 'mini-combinatorial library' of closely related compounds composed of biochemically related analogues, precursors and catabolites that may have overlapping pharmacological activities. For example, while genestein is the best known soybean isoflavonoid with reported anti-cancer and cardiovascular benefits [43], detailed biochemical analysis of soybean concentrate revealed the presence of at least 12 structurally similar isoflavones (Table **4**) [44] in addition to their multiple biochemical precursors. Many of these compounds were shown to exhibit related pharmacological activities that act together to produce the overall cytotoxic effects of soybean extracts on cancer cells. [43, 44].

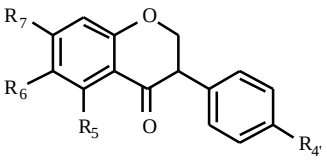
Mini-combinatorial libraries stored in each plant contain molecules that may be too difficult for a synthetic chemist to make. Complex glycosylation reactions carried out by dozens of glycosyltransferases present in each plant [45, 46] and highly specific hydroxylations carried out by highly

diverse plant cytochrome P450 monooxygenases [47] are often too complex for human chemists to perform. In addition, human chemists cannot yet compete with the complex stereochemistry carried out by plant enzymes [48]. It is estimated, however, that natural products, particularly those made by plants, tend to have fewer nitrogen, halogen, or sulfur atoms than synthetic pharmaceuticals [49]. Therefore, many structure activity relationship programs experiment with adding halogen or sulfur atoms to natural products in attempts to convert them to synthetic drugs. A hybrid approach that begins with a specific natural product(s) isolated from a plant that is subsequently derivitized through the synthetic combinatorial approaches is now being considered as a valuable NCE discovery strategy for the future [1, 50].

Table 4. Structures of Isoflavonoids Present in Soybean Seeds. Adapted from [44]



$R_{4'}$	R_5	R_6	R_7
OH	H	H	OH
OH	OH	H	OH
H	H	OCH_3	OH
OCH_3	OH	H	OH
OH	H	H	O-Glc
OH	OH	H	O-Glc
H	H	H	O-Glc
OH	H	H	6”-O-Ac-Glc
OH	OH	H	6”-O-Ac-Glc
OCH_3	H	H	2”,6”-O-di-Ac-Glc



$R_{4'}$	R_5	R_6	R_7
OH	H	H	O-Glc
OH	OH	H	O-Glc

THE POWER OF ELICITATION

The complexity and unpredictability of the health effects of botanical therapeutics does not end with the pleiotropicand interacting effect of their components. It is well known that different stresses, locations, climates, microenvironments and physical and chemical stimuli (often called elicitors) qualitatively and quantitatively alter the content of bioactive secondary metabolites. This is particularly true for phytochemicals that are well documented for their biological activity, such as alkaloids [51], phenylpropanoids [52] and terpenoids [53, 54], whose levels may increase by 2 to 3 orders of magnitude following stresses or elicitation [55, 56]. Stress-mimicking chemical elicitors also increase the amounts of natural products widely used as pharmaceuticals, such as taxol [57, 58], tropane alkaloids [59], indole alkaloids of *Catharanthus roseus* [60, 61] and salicylates [62]. Recently we demonstrated the effect of elicitation on the production of biologically active phytochemicals in the roots of hydroponically cultured plants [2]. Root extracts produced from 588 elicited and non-elicited plants were screened in anti-cancer assays. Data indicate that 64% of plant species (76 out of 119) would have been missed during the more conventional bioprospecting activity, leaving only 43 species active in the non-elicited state as potential sources of anti-cancer leads. The percentage of missed leads would be even greater if only one cancer cell line was used for screening (79% for breast cancer, 72% for melanoma, and 77% for lung cancer). Elicitation had a similar stimulatory effect on the production of anti-microbial compounds in plants [2]. As a specific example, the genistein content of yellow lupine roots was increased by an order of magnitude by the addition of elicitors to the hydroponic medium supplied to the plant [63]. Similarly, levels of salicylic acid (probably the first purified and structurally characterized therapeutic agent from plants) increased dramatically in plants infected by viral or bacterial pathogens [62, 64]. Just as tomatoes from different gardens taste differently and the same grapes produce distinctly flavored wines each year, plants as sources of botanical therapeutics may change their biochemical composition and medicinal properties unless their growing environment is strictly controlled. This simple fact has a significant implication for the discovery and manufacturing of botanical therapeutics that need to be carried out in the conditions that favor the biosynthesis of pharmacologically active compound(s).

DISCOVERY OF BOTANICAL THERAPEUTICS

Today’s pharmaceutical discovery is characterized by the dominance of high throughput *in vitro* ‘bind and find’ approaches and declining interest in phytochemical bioprospecting. Thus, relatively few efforts are currently being directed to identifying technologies that are better suited for discovering botanical therapeutics. The successful development of the next generation of botanical therapeutics, possibly hiding in our grocery stores, fields, greenhouses, forests, meadows and deserts [10, 65], may require more sophisticated approaches and better acceptance of the concept of multi-component therapeutics.

Ethnobotanical bioprospecting, which takes advantage of traditional medicinal knowledge, and random ‘grind and find’ bioprospecting have been two methods of choice for phytochemical drug discovery. However, in recent years the development of novel botanical therapeutics from ethnobotanical sources fell short of expectations [66]. It can even be argued that the NCE-driven ethnobotanical approaches practiced throughout human history have already identified

the most obvious single-component botanical therapeutics (Table **1**). However, this lack of success may also be attributed to relatively simplistic and reductionist approaches practiced by ethnobotanical explorations that emphasized isolation of a single active ingredient. Little care has been taken in re-growing plants in conditions that stimulate the production of bioactives or focusing on the potential interactions of the phytochemicals in producing the overall therapeutic effect. In addition, disproportional effort has been placed on relatively hydrophobic phytochemiclas that can be extracted with organic solvents. Relatively small effort was placed on water-soluble phytochemicals with potential medicinal use. Hopefully, future botanical explorations will be more cognizant of these factors, thus maintaining ethnobotanical bioprospecting as a valid discovery strategy.

One of the approaches we have recently developed as a biorational discovery tool for botanical therapeutics is called Reversed Structural Bioinformatics (RSB). This approach uses computational approaches to uncover phytochemicals that structurally resemble synthetic molecules effective against certain clinical targets. Synthetic compounds that interact with certain protein targets can be analyzed to define the ideal pharmacophore(s) [67] that can then be referenced against known structures of phytochemicals. The most useful databases that include phytochemical structures are Chapman and Hall Dictionary of Natural Products (http://www.chemnetbase.com/scripts/dnpweb.exe), Chemical Abstracts Databases (http://www.cas.org) and Beilstein and Gmelin CrossFire Databases (http://chemistry.library.wisc.edu/beilstein/home.htm). These databases can be used as a foundation for the more complex interactive databases of phytochemical structures. Plants containing the natural analogs of synthetic bioactives identified through RSB are grown, elicited, if necessary, putative bioactives extracted and their activity validated in *in-vitro* and cellular screens. This approach forgoes the need for chemical synthesis of putative leads and relies instead on the tens of thousands of compound-strong libraries stored inside green plants – some of the best chemists living on earth. The identification of gaultherin (a conjugated salicilate from wintergreen, *Gaultheria procumbens* L.) as a potential alternative to aspirin with reduced potential for gastrointestinal irritation and ulceration [68] is a good example of the application of the RSB approach to the discovery of botanical therapeutics.

Changes in gene expression are important in many biological processes, such as the onset and progression of human diseases. Until recently these changes were difficult to study particularly when multiple genes were affected simultaneously. The progress of molecular biology now allows effective simultaneous monitoring of the effects of therapeutic agents on transcription of multiple disease-associated genes, using Real Time reverse transcription polymerase chain reaction (RT-PCR) technology pioneered in 1993 [69] and microarray (gene-chip) strategies [70, 71]. While the latter is still too expensive and cumbersome to be generally useful as a discovery method, the former provides an effective discovery tool for the more biorational, lower-throughput discovery approaches.

Microarray technology may also allow identification and cloning of genes encoding the biosynthesis of bioactive phytochemicals in wild plants for subsequent transfer to the cultivated species. Application of the above high-throughput genomic tools to nutritional research and medicinal food development is called nutrogenomics [72], a term commonly used but poorly defined.

Real Time RT-PCR is particularly useful for studying the effects of multi-component botanical therapeutics in intact cells. Real time RT-PCR-based assays are uniquely compatible with biological extracts, since they measure the effects of compounds in living cells rather than *in vitro* binding or enzymatic systems that usually utilize isolated target proteins. Extracts often produce artifacts in such *in vitro* systems (see above). Cell-based systems, while cumbersome to use in a high throughput format, are much less prone to the artifacts associated with multi-component mixtures. Real time RT-PCR technology also permits studying the effects of various agents on the expression of selected genes in animal organs and tissues [73].

Just as Real Time RT-PCR, whole-cell high-content screening approaches [74] go beyond the affinity-binding information obtained through high-throughput screening. High-content screening uses complex cellular assays that monitor the effects of molecules on many morphological and functional parameters documented with imaging/pattern recognition technology and analyzed with informatics software. This emerging discovery technology dovetails nicely with the needs of identifying the pharmacological effects of complex mixtures of phytochemicals.

DEVELOPMENT OF BOTANICAL THERAPEUTICS

Multi-component botanical therapeutics also present unique challenges in identifying their active ingredients and in validating their clinical effects. Activity-guided fractionation and reconstitution experiments currently used to characterize compound interferences within a mixture are cumbersome and time consuming. Clinical confirmation of the efficacy of multi-component botanical therapeutics proved to be an elusive and complex goal. For example, despite at least 83 clinical trials, our understanding of the potential health effects of phytoestrogen-containing foods and supplements is far from complete [43]. Factors that contribute to this lack of understanding include the functional and structural diversity of phytoestrogens in tested preparations, variability in their content from different batches of plant materials, inconsistent use of extraction methods and formulations, and interference from other compounds present in various phytoestrogen sources. Similar factors interfere with the final clinical conformation of the effects of *Echinacea*, *Gingko biloba* and St. John's Wort (see below). Undoubtedly, many of these factors would not exist for clinical trials involving a single NCE.

MANUFACTURING OF BOTANICAL THERAPEUTICS

Public distrust of dietary supplements and some functional foods is justly fueled by reports of the presence of adulterants [75-78], variations in the amount of active ingredients [79, 80], safety concerns and unproven health benefits. Sales of ephedra, one of the most popular dietary supplements, were recently banned by FDA which has never

before ordered a dietary supplement to be pulled from the market for safety reasons. Ambiguous clinical data continue to plague these products. For example, contrary to the earlier studies, a recent large clinical trial of *Echinacea* showed that, not only was it ineffective in reducing upper respiratory tract infections in children, but actually increased the incidence of skin rash [81]. Similarly a recent well-designed human study of *Gingko biloba* extract has shown no effect on age-associated memory impairment [82] while others showed much more promising results [83]. Such discrepancies can be explained by the inherent difficulty in achieving functional and biochemical consistency between batches of botanical therapeutics prepared and administered at different times. While chromatographic analysis is often employed to produce biochemical fingerprints used for product comparison [84], the apparent similarity in the biochemical fingerprints between batches does not prove pharmacological equivalence. Actually, in the absence of information about the identity of active ingredients, such analysis is hardly reliable, since chromatography provides an incomplete picture of the qualitative and quantitative composition of a complex extract.

The inclusion of functional assays in quality control protocols performed by the manufacturer, in addition to chromatographic analysis, will provide a partial solution to assuring batch-to-batch consistency. It is also important to strictly monitor the condition at which the source plants are grown, harvested and extracted in order to avoid environmentally imposed variations in their chemical composition. In some cases elicitation may be used to increase the content of pharmacologically active ingredients in botanical therapeutics. While difficult to administer in the field, environmentally benign elicitors [2] can be simply added to the nutrient medium circulated in commercial hydroponic greenhouses. Techniques of somaclonal propagation that allow asexual production of large quantities of genetically identical plants, can further reduce variability and rapidly multiply individual plants enriched in medicinal phytochemicals. Novel reliable and enforceable quality control approaches to manufacturing and cultivation of botanical therapeutics have to be established, since issues associated with their production differ from the issues associated with the manufacturing of single active ingredient drugs.

FUTURE

People ingest a vastly greater diversity of pharmacologically active chemicals in the form of foods than as drugs, often not realizing that many drugs were derived from the compounds originally discovered in foods. The 20th century introduced a clear separation between drugs and foods where drugs became primarily synthetically manufactured components of pills and capsules while foods retained their character as naturally-produced mixtures of compounds presented on the plate. Consumers learned that they could eat unhealthy foods and then at least partially correct their indiscretions by swallowing a synthetic pill. Extracts, powders and potions used by herbalists and shamans were officially reincarnated in the form of botanical nutraceuticals. What will come next?

It is likely that plants' contribution to future medicine will move beyond the realm of NCE discovery into the next generation of multi-component botanical therapeutics delivered as functional foods, both standard and individually tailored; scientifically designed, optimized, standardized and validated dietary supplements and botanical drugs. Major technological improvements in the ways we discover, develop and manufacture botanical therapeutics, assisted by a favorable regulatory environment will be required to achieve this transition. It is yet unclear whether pharmaceutical companies, food companies or chemical-life science companies will market these innovations or whether the area will develop as an independent industry from fledgling biotechnology companies. Yet, it seems that the pleiotropicc-linical effects that may be achieved by the interacting components of botanical therapeutics are slowly gaining serious attention by the scientific and regulatory community. Foods, beverages and extracts with medical claims are moving onto the shelves of grocery stores in greater numbers each year. Plant scientists are now breeding crops for the greater amounts of antioxidants, carotenoids, vitamins, flavonoids and other therapeutically active compounds. Creating plant varieties with enhanced nutritional and medicinal qualities will become a much larger component of private and public breeding programs. Metabolic engineering, while still on the fringes of public acceptance and in search of methods for cloning and transformation of complex biochemical pathways, will soon be able to augment our crops with a much greater variety and quantity of pharmacologically active compounds than can be achieved through conventional breeding or elicitation [85]. As an added benefit, the production of these pharmacologically active compounds in plants will likely be done in a sustainable manner, while adding substantial value to agriculture and food processing.

Multi-component botanical therapeutics may provide an effective delivery vehicle for the prevention of genetic and life-style associated diseases. Individualized functional diets and supplements prepared from specially cultivated plants and recommended by a physician may reduce the incidence of disease, providing that the future consumer can mentally and physically reconnect plant-derived foods and health. On the other hand, FDA-approved and physician prescribed or recommended botanical drugs, may help to eventually replace botanical nutraceuticals and assure much greater levels of consistency, safety and regulatory compliance. Their presumed ability to deliver concentrated and optimized mixtures of interacting compounds may effectively supplement existing single component drugs, providing that care is taken to understand and manage potential interactions between these groups of pharmaceuticals.

While, the first generation botanical therapeutics go back to ancient times, their modern successors are just emerging from the proof of concept stage, having a somewhat shaky reputation with many scientists, pharmaceutical companies and regulatory agencies. Clearly, many technologies required for the successful discovery, development and production of botanical therapeutics are not yet in place, and efforts required for their emergence may be substantial. Yet if successful, these efforts may result in the partial merger of grocery stores and drug stores as well as healthier and greener planet.

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