Introduction

Plants have been important in healing human disease for millennia. Herbal medicines have served human cultures worldwide, and have also been important sources of modern pharmaceuticals. Specific phytochemical groups from higher plants have been the sources of the bulk of natural product drugs used in biomedicine (Raskin et al., 2002). The plant-derived alkaloids that are widely used as drugs include atropine and the related hyoscyamine and scopolamine; camptothecin (used in synthesizing important cancer chemotherapeutics); cocaine; codeine, morphine, and other opiates; colchicine; emetine; galanthamine; nicotine; physostigmine; pilocarpine; reserpine; tubocurarine; quinidine and quinine; vinblastine and vincristine; and yohimbine. Important plant-derived terpenes and steroids include artemisinin; diosgenin, hecogenin, and stigmasterol, used in synthesis of important steroid drugs; taxol and other taxoids, sources of cancer chemotherapeutics; and digoxin and digitoxin. Phenolics include the perva-sive and versatile aspirin, podophyllotoxin (used in synthesis of the cancer drug etoposide), and sennosides A and B. Also important are mixtures and extracts that are licensed as pharmaceuticals, such as ipecac and senna.

As important as plants have been in the history of drug development, they are currently losing the competition in discovery of new chemical entities for drug discovery. Because of novel approaches to sourcing of lead compounds, and also the political issues regarding use of genetic resources that have been raised by the Convention on Biodiversity, interest in plant-based bioprospecting among the large pharmaceutical manufacturers is significantly reduced. There has been an evolution in the sources of materials used for screening that has paralleled the evolution of screening technologies. While humans have been involved in what is essentially bioprospecting for at least 10,000 years, chemical synthesis as a source of potential drugs has been in use for 100 years; combinatorial chemistry has been used for the...
last 20 years; while target-specific computational design has been in use for only 10 years. These more recent synthetic methods of accessing materials for screening are much better adapted to the needs of the high throughput screens currently used by pharmaceutical companies than the slower-paced process of bioprospecting.

As important as new chemical entities are in drug development and the management of human health and disease, they are not the only ways that plants can be used to address health concerns, even in the highly regulated environment of the United States. The regulatory pathways to the use of plants in human health have also been evolving, and now include the following:

- Drugs (new chemical entities): single compounds intended to cure or prevent disease. Regulated through Food and Drug Administration (FDA) approval based on Investigational New Drug (IND) status and a New Drug Application (NDA). An example is Taxol (paclitaxel).
- Botanical Drugs: extracts of plants intended to cure or prevent disease. Regulated through FDA approval based on IND and NDA. Over 150 INDs have been filed for botanical drugs, with ongoing clinical trials, but few have yet received approval.
- Dietary Supplements (nutraceuticals): extracts of plants intended to supplement the diet. Health claims (structure–function claims) are possible but not disease claims. Regulated by the FDA based on premarket notification or history of use. Examples are garlic extract or milk thistle extract.
- Generally Regulated As Safe (GRAS) food ingredients or additives: extracts which affect food characteristics. Health claims are possible (separate regulation enables health claims to be made for foods). Regulated by the FDA based on self-affirmation by experts or common use in the food supply prior to 1958 and are subject to premarket FDA approval. Examples are marigold extracts or phytosterols.
- Cosmeceuticals: extracts which supplement cosmetic products. No health claims are associated with them and there are no FDA regulations. Safety is established by the manufacturer. An example is aloe cream.
- Recombinant Proteins: pharmaceutical proteins expressed and isolated from plants. Regulated by FDA approval based on IND and NDA. Additional regulations by the US Department of Agriculture and Environmental Protection Agency concern production of such products. Several are in clinical trials but none has yet been commercialized.

The designation of the class of “Botanical Drugs” is only the most recent step in the US adoption of multicomponent or plant extract drugs. In the 1930s, Sarracenia purpurea (pitcher plant) extract was sold under the name of Sarapin. Lilly sold a preparation of phytosterols, Cytellin, in the 1950s. Premarin (conjugated estrogen tablets), by Wyeth, a heterogeneous drug based on equine urine, is one of the most widely prescribed drugs in history, as part of postmenopausal hormone therapy. Over-the-counter drug monographs on psyllium (Metamucil), senna extract (Senokot), and ipecac (ipecac syrup) subsequently resulted in successful drug products. It was on June 9, 2004, however, that the FDA approved the Botanical Drug Guidance that has fundamentally changed the status of plant extract drugs in the United States, setting out a clear path to regulatory approval as drugs for treatment and prevention of disease for these complex entities. Notable among the INDs filed in response to the Guidance that have received drug approval is the first US botanical drug, the green tea extract Polyphenon E (MediGene), indicated for genital warts, approved in 2005. In the same year Canada approved the Bayer HealthCare product Sativex, a cannabis extract for multiple sclerosis pain.

Botanical drugs as defined by the FDA are intended for the diagnosis, cure, mitigation, treatment, or prevention of disease in humans. This is in contrast to dietary supplements, which are specifically not intended for these objectives but rather for the support of normal body functions or structures. Botanical drugs consist of vegetable materials, which may include plant materials, algae, microscopic fungi, or combinations thereof. They may be made available as solution (e.g. tea), powder, tablet, capsule, elixir, topical or injection, or other forms. They often have unique features such as complex mixtures, lack of a distinct active chemical compound, and substantial prior human use. Fermentation products, single new chemical entities derived from plants, and highly purified or chemically modified botanical substances are not considered botanical drugs. Although they may contain multiple chemical components, botanical drugs are not considered “combination drugs” by the FDA, a classification restricted to products composed of two or more single compound drug entities which are governed under a complex regulatory framework to prevent harmful pharmacokinetic interactions.

Botanical drugs, as multifunctional, multicomponent mixtures, have specific potential advantages in health care, especially for the management of chronic degenerative diseases that are prominent in Western societies. Their multitargeted mode of action implies a special adaptation to the multiple physiological derangements characteristic of chronic and metabolic diseases. The multiple components may have additive or synergistic effects on these targets. The bioactives that characterize botanicals may have been perfected through evolution to react with physiological targets. Because there are multiple components, there may be
Botanicals for chronic diseases

We focus here on two botanicals in developing botanical drugs for chronic diseases. *Tripterygium wilfordii* Hook.f. (Celastraceae) or Thunder God Vine is currently in clinical trials as a botanical drug for rheumatoid arthritis. *Artemisia dracunculus* L. (Asteraceae) or Russian tarragon is in an earlier stage of development: animal studies are elucidating mechanisms of action and formulation properties.

These two phytomedicinals have been developed through collaborations with both the Central Asia International Cooperative Biodiversity Group (ICBG) based at Rutgers University and the National Institutes of Health Center for Dietary Supplements Research on Botanicals and the Metabolic Syndrome, as well as Phytomedics, Inc. The Central Asia ICBG, titled “Building New Pharmaceutical Capabilities in Central Asia,” is a collaboration of Rutgers University with institutions in Kyrgyzstan and Uzbekistan, with Principal Investigator Ilya Raskin, PhD. The goal of the program is to facilitate development of the natural product-based pharmaceutical capabilities in the host countries while encouraging biodiversity conservation and exploration, developing sustainable harvesting practices and enhancing international collaboration and training. A long-standing collaboration between Rutgers University and the Uzbekistan and Kyrgyzstan institutions is the basis for this ICBG, which is supported by legal agreements to ensure equitable benefit-sharing and compliance with biodiversity treaties. The National Institutes of Health (NIH) Botanical Center for the Metabolic Syndrome, Principal Investigator William Cefalu, PhD, is based at the Pennington Biomedical Research Center of Louisiana State University in Baton Rouge, LA, and collaborates with the Center of Agriculture and the Environment at Rutgers University. Phytomedics, Inc., based in Jamesburg, NJ, is involved in the development of several botanical drugs, and is concentrating on development of innovative means of quality assurance and standardization of botanicals in addition to the requisite preclinical and clinical steps of drug development.

**Thunder God Vine extract**

*T. wilfordii* is a perennial vine-like plant and has a history of use in China for inflammatory and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, Behcet’s disease, and psoriatic arthritis. A well-known and significant toxicity profile (LD<sub>50</sub> in mice as low as 160 mg/kg) can be addressed by removing the outer stem bark layer of the roots and extracting it with ethanol followed by ethyl acetate partitioning (LD<sub>50</sub> in mice 860–1300 mg/kg) (Lipsky et al., 1996). An extract based on this extraction method, PMI-001, has been investigated for use in rheumatoid arthritis.

The compounds triptolide (1) and tripdiolide (2) are responsible for a large part of the bioactivity of PMI-001 (Figure 1). Close to 400 compounds have been isolated from *Tripterygium* species, 95% of which are terpenoids, including the diterpenoid epoxides 1 and 2, the most abundant and effective bioactives of *T. wilfordii* (Ma et al., 2007). Other bioactives in PMI-001 include tripterygiol, (−)-syringaresinol, triptolide, euonine, wiforine, cangrinine, and 3,4,5-trimethoxyphenol.

The main activities of the extract and the bioactive compounds involve transcriptional inhibition of proinflammatory genes including interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (i-NOS), interleukin-1B (IL-1B), and cyclooxygenase-2 (COX-2). The extract also has a steroid-sparing effect, reducing the need for the corticosteroid prednisone when co-administered with it in clinical trials. The extract also has shown a better side effect profile than conventional steroids in clinical trials.

The long history of human use and preclinical and clinical trials all suggest that *T. wilfordii* extracts may be used safely. In studies in which various extracts were compared with conventional rheumatoid arthritis (RA) drugs such as methotrexate, the extracts generally had fewer side effects, although in studies with some extracts up to 30% of subjects reported side effects (Yao & Nian, 2004), indicating the need to carefully prepare and formulate these extracts. Antifertility effects have been observed in men administered the extracts, a side effect that might be explored as a lead for male contraceptive drugs (Brinker et al., 2007).

![Figure 1. Structure of triptolide (1) and tripdiolide (2).](image-url)
The mode of action of 1, related compounds, and the extract PMI-001 is not conclusively understood. However, the following preliminary hypothesis is consistent with experimental observations and explains the anti-inflammatory, immunosuppressive, and steroid-sparing effects observed in studies of rheumatoid arthritis (RA). T-cells and other monocytes in the synovial membranes of the joints become overactivated in RA. Both PMI-001 and 1 bind to the glucocorticoid receptor (GR) in T-cells. This binding prevents the activation of genes in the glucocorticoid response element. This is in contrast to corticosteroid medications such as dexamethasone, which bind to the GR, but still can activate GR-responsive genes that produce the typical steroid effects such as hypoglycemia, weight gain, osteoporosis, and suppression of pituitary-adrenal function. This accounts for the reduced risk of side effects of the extract relative to steroid medications. The complex of the extract and the GR, however, still may inhibit the activation of proinflammatory transcription factors such as nuclear factor kappa-B (NF-κB) and activator protein-1 (AP-1). This would reduce the transcription of other proinflammatory genes. Triptolide is also known to inhibit TNF-α. TNF-α also activates NF-κB, and this is the route by which 1 and PMI-001 are thought to inhibit IL-1, i-NOS, and COX-2, resulting in strong anti-inflammatory and immunosuppressive effects in addition to the steroid-sparing effect and improved side effect profile (Brinker et al., 2007; Ma et al., 2007).

Further in vitro characterization of the mode of action using polymerase chain reaction (PCR) arrays uncovers more detail about this mode of action. Among the genes affected by PMI-001 extract are CD14, TLR4, and TLR7. These genes are all involved in the innate immune system and take part in detecting bacterial lipopolysaccharides. Downstream effects of reduced activation of these genes are reductions in apoptosis, the mitogen activated protein kinase (MAPK) signaling pathways, and the transcription factor NF-κB. Reduction in MAPK signaling results in reduced expression of AP-1, as well as IKKB, which also stimulates activity of NF-κB. The reduction in activity of AP-1 and NF-κB results in decreased synthesis of the inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8. Reduction in NF-κB activity also decreases CD80, CD40, and CD86. These three proteins are found on B-cells and monocytes, and stimulate the activity of T-cells, which are involved in rheumatoid arthritis. Reduced expression of these proteins would tend to decrease the overactivity of T-cells involved in RA.

To date, three successful human clinical trials have been carried out with PMI-001, under individual investigator IND #39,191 (although PMI-001 is a botanical and is following a drug development pathway, the IND was obtained prior to the Botanical Drug Guidance of 2004). Of the two published trials, Tao et al. (2001) reported a Phase I study of the extract that began in 1993 in 13 patients with established RA. Nine patients tolerated the extract in doses of up to 570 mg/day, although three withdrew early in the trial, receiving only 180 mg/day maximum. Only one withdrawal was related to an adverse effect, in a patient who developed diastolic hypertension at a dose of 180 mg/day extract. Six of 10 subjects showed disease improvement at doses of 180 mg/day, while eight of 10 subjects who received over 360 mg/day experienced improvement in both clinical and laboratory findings. One patient experienced remission.

A prospective double-blind placebo-controlled trial was conducted in RA patients in whom conventional therapy had failed (Tao et al., 2002). Subjects were randomized to receive placebo, low dose (180 mg/day) or high dose (360 mg/day) extract for 20 weeks, after which an open-label observational period was instituted. Of the 35 subjects enrolled in the trial, 21 completed the 20 week study. One patient in each group experienced side effects that precipitated withdrawal. Among the subjects who completed at least 4 weeks of treatment, eight in the high-dose and four in the low-dose group experienced clinical response. Fifteen subjects were enrolled in the open-label intervention, of whom 11 experienced response. Diarrhea was the most common side effect and resulted in one withdrawal in the high-dose group; there were no withdrawals due to adverse effects in the open-label extension.

An important part of the development of PMI-001 is quality control and standardization. These are carried out on both biochemical and functional levels. Quantitative biochemical high performance liquid chromatography (HPLC) fingerprint techniques are used to standardize the amounts of 1 and 2 in the extract. Quantitative real time reverse transcriptase-polymerase chain reaction (RT-PCR) is used to check effects of the extract on IL-2, TNF-α, i-NOS, and COX-2, while enzyme-linked immunosorbent assay (ELISA) testing is used to monitor effects on IL-1 and IL-2. Successful standardization, however, depends on an adequate supply of consistent raw materials. In this respect, systematic efforts have been made to ensure a sustainable supply of such materials in the case of PMI-001. T. wilfordii was originally wildcrafted from sources in China, but this is obviously not a long-term solution to the problem of developing a standard botanical drug. Genetic materials from several areas of China were collected and evaluated to determine the best varieties. Selected varieties were then clonally propagated to obtain genetic uniformity. The selected lines were propagated in controlled greenhouse environments, followed by intensive field cultivation. An additional critical step in production of a consistent product is standardization of harvesting and processing of harvested materials, which have also been developed. Avoidance of contaminants is one goal of standardized...
harvesting and processing. Finally, Good Manufacturing Practices (GMP)-based industrial processing has been developed, the final step in producing a consistent extract suitable for further drug development.

**Russian tarragon**

A second botanical drug that is currently in development is an extract of *A. dracunculus* (PMI-5011). Species of *Artemisia* have been used traditionally for diabetes in Iraq, India, Turkey, and Britain. *A. dracunculus* was selected by the Rutgers University Biotech Center from screening of extracts to decrease insulin resistance, and an ethanolic extract of the plant has been investigated as part of the Botanical Center grant for potential activity in type 2 diabetes. Special features of the plant material investigated include the hydroponic cultivation of the plant under strictly controlled conditions and the extraction of material from the fresh shoots of the herb to preserve the active components of the plant (Cefalu et al., 2008). The species is already in cultivation, and a commercially available seed variety was used to produce this material.

Assay-guided fractionation elucidated six flavones and chalcones that contribute to the bioactivity of the plant, using *in vitro* bioassays with *in vivo* confirmation. The active compounds of most interest are the following: 4,5-di-O-caffeoylquinic acid, davidigenin, 6-demethoxycapillarisin, 2′,4′-dihydroxy-4-methoxydihydrochalcone, 2′,4′-dihydroxy-4′-methoxydihydrochalcone, and sakuranetin. The enzymatic targets of these compounds are aldose reductase, protein tyrosine phosphatase-1B (PTP-1B), and phosphoenolpyruvate carboxykinase (PEPCK).

The safety information on this species was limited to historical records of human use as a culinary herb, and the Botanical Center thus undertook extensive 90-day toxicologic investigations in animal studies. There were no signs of toxicity in body weight, motor activity, or results of a functional observational battery. Gross necropsy and clinical chemistry did not indicate any effect on organ mass or blood chemistry. No lesions associated with the extract were found on microscopic examinations of tissues of animals dosed with the extract (Cefalu et al., 2008). The Ames test for mutagenicity was negative. The extract thus appears to be safe for further clinical trials.

A 2006 study on application of the extract in diabetic mice observed lowering of blood glucose and insulin levels in mice that were genetically diabetic (Ribnicky et al., 2006). Additionally, in diabetes induced by streptozocin (STZ), the extract also lowered blood glucose by 20%. The activity in STZ-induced diabetes suggested that the extract reduced blood glucose in animals with impaired production of insulin due to destruction of the pancreas by STZ treatment. This indicates that the mechanism of action of the drug is not likely to be stimulation of insulin production, which is the mechanism of the sulfonylurea-type antidiabetic drugs. The mechanism is more likely to be based on promotion of insulin effects in peripheral tissues such as liver or muscle. The effectiveness of the extract in genetically diabetic mice, which spontaneously develop diabetes as they age, with high blood glucose and insulin levels, suggests that an effect on insulin resistance is likely. This suggests a mechanism involving enhancement of glucose uptake from muscle or adipose tissue and possible reduction of glucose output. *A. dracunculus*, therefore, appears to have multiple mechanisms of action, consistent with the multiple bioactive compounds.

An effort was thus made to determine the specific mechanisms through which the extract may act in muscle and liver. Various possible mechanisms have been studied in detail, confirming multiple mechanisms, in both muscle and liver. In muscles, PMI-5011 increases cellular signaling through the insulin receptor pathway by decreasing levels of PTP-1B, an enzyme that inhibits insulin signaling and thus promotes insulin resistance. It also enhances activity of the gene insulin receptor substrate-2, also involved in insulin signaling. These effects may tend to improve insulin function in muscle cells, and indeed, PMI-5011 is found to enhance the ability of insulin to promote glucose uptake in cell cultures of human skeletal muscle. The extract also increases glycogen synthase, glycogen accumulation, and fatty acid oxidation, additional activities that indicate improvement in metabolism of carbohydrates (Cefalu et al. 2008). In the livers of diabetic animals and in cultured hepatocytes, PMI-5011 reduces expression of PEPCK, an enzyme that catalyzes the process of gluconeogenesis or glucose synthesis. The reduction of this function results in a decrease in glucose output from the liver, which may contribute to overall lowering of blood glucose. A human trial in insulin resistance is currently under way and has had a positive interim data analysis. These analyses also suggest multiple mechanisms of action based on multiple active compounds, and represent one of the potential strengths of botanical drug development.

Standardization and quality control of a botanical drug with six bioactive compounds poses challenging problems. In addition to biochemical standardization, however, the formulation of the extract to promote bioavailability of the multiple bioactive compounds has been explored as a means of maximizing the potential for effective blood levels of the compounds. Many bioactive compounds from plants are poorly water soluble, resulting in poor bioavailability. One way to improve the bioavailability of poorly soluble compounds is to formulate them with bioenhancing agents commonly...
based on fats. Several such bioenhancing agents are used in formulation of conventional drugs, and their application was also studied as a way of improving bioavailability of PMI-001 in mice. Labrasol (Gattefosse Corporation) consists of glyceride esters of polyethylene glycol and fatty acids, and is used to enhance absorption and bioavailability of antibiotics. Labrasol and other agents including Labraf il and dimethylsulfoxide (DMSO) were used to formulate doses of PMI-5011 and metformin given to mice (Ribnicky et al. 2009). Diabetes was induced in the mice by feeding very high fat diets. PMI-001 was given to mice by gavage at a dose of 500 mg/kg and the concentration of active compounds was assessed through ESI-LC-MS (electrospray ionization-liquid chromatography-mass spectrometry). Marked differences in concentration of the active compound sakuranetin were observed in comparing the different formulation agents, with sakuranetin concentrations twice as high when given with Labrasol as when formulated with DMSO or Labraf il. Labrasol also promoted the greatest hypoglycemic activity of the different agents tested. In testing of a 1:2 mixture of water–Labrasol as a formulation agent for PMI-001, blood glucose was reduced by nearly 50% in genetically diabetic mice given doses of 200 mg/kg of the extract after 3 days of dosing.

**Conclusion**

The work of the Botanical Center and the ICBG program, in cooperation with the research at Phytomedics, Inc., demonstrates several of the most relevant aspects of botanical drug development. Botanical extracts typically include multiple active components and are derived from living organisms. To develop a botanical as a drug (or, for that matter, as a dietary supplement), standardization must take place. There are three pillars of multicomponent botanical standardization – manufacturing, biochemical, and functional standardization. Manufacturing a product free of adulterants and contaminants begins before plant material arrives at the laboratory or manufacturing plant. In nature and in traditional use of botanicals, variability in the genetic makeup of a species, and the variation in environments in which it may grow or be cultivated, lead to variable biochemical characteristics. A primary concern in producing botanical drugs, therefore, is to develop a sustainable and uniform means of cultivating plant source materials. This was demonstrated in the production of *T. wilfordii* extract, based on plants sourced in China and selected for the most suitable germplasm, followed by greenhouse and field cultivation, with improvement of harvesting and processing techniques. Hydroponic cultivation of *A. dracunculus* is being used to produce extracts for animal studies.

Having obtained consistent plant material, further multicomponent standardization must be carried out for botanical drugs. Biochemical standardization is used to optimize contents of bioactive compounds and avoid toxic compounds. Beyond simple chemical fingerprinting, formulation properties of multicomponent botanical extracts should be explored to investigate the bioavailability of the major active compounds. Functional standardization includes both observation of *in vitro* bioactivity and *in vitro* and *in vivo* investigation of modes of action. Such investigations were carried out in both *T. wilfordii* and *A. dracunculus*. Understanding of the modes of action can assist in determining optimal standardization by highlighting the major biochemical pathways that are crucial to the activity of the drug. Botanical drugs can affect these pathways at multiple target points, due to multiple active compounds, as was highlighted in the research on *A. dracunculus*.

The research on botanical drugs carried out by the Botanical Center for the Metabolic Syndrome, the Central Asia ICBG, and Phytomedics is yielding valuable advances in models for producing multicomponent drug products. Because of their inherently multitargeted modes of action and their relative safety, such drugs may contribute much to the health care of the future, especially in the area of complex chronic diseases, which seem destined to afflict populations worldwide for decades to come.

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