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The Development of Botanical Drugs – A Review

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Abstract

This manuscript has presented evidence for the value of and strategy for the development of Plant (Botanical) preparations as approved drugs (pharmaceuticals). Such preparations have historically been the basis for many (most) traditional medicines but have not usually been developed clinically in such a way as to provide confirmation of their safety and efficacy and importantly their consistency such that they provide predictable clinical outcomes patient to patient and reproducibly over time. We outline the process by which these attributes may be accomplished. There are numerous unmet medical needs where botanical drugs can contribute to their solution.

Keywords: Botanical drug development; Entourage effect; Compound synergy

Introduction

Plants for millennia have provided the major source of the medicinal compounds used to treat our illnesses. Until the early 1900's plant-derived pharmaceuticals, primarily as preparations of mixtures derived directly from plant material, represented the major source of pharmaceuticals throughout the world. Even today, according to the World Health Organization (WHO), roughly two-thirds to three-quarters of the world's population relies upon medicinal plant preparations for their primary health care needs. The recognition of the importance of active ingredients in these complex mixtures fostered the development of organic chemistry, physiology, and pharmacology as important contributors to the practice of medicine. With the isolation and characterization of the structures of select natural products, synthetic organic chemistry became an important contributor to pharmaceuticals and a number of important pharmaceuticals modeled upon natural products began to be developed. The development of aspirin based upon the salicylic acid of willow bark is a classic example. Testing of numerous chemical compounds against an important parasitic disease and observation of efficacy led Paul Ehrlich to postulate "the Magic Bullet" concept in the early twentieth century [1,2]. In the 1930s, the observation that microorganisms produced substances which inhibited the growth and development of other microorganisms triggered the "Age of Antibiotics" and their use in medicine [3]. These naturally occurring substances had a profound impact upon humankind through the adequate treatment of infectious diseases, formerly a major cause of early mortality in humans. Consequently, significant increases in life span have occurred over the last 100 years. The efficacy and safety of these newly discovered medicaments further solidified our belief in the magic bullet paradigm [4]. The utilization of microorganism fermentation to produce natural products for application in medicine and the growing sophistication of synthetic organic chemistry largely displaced interest in plant-derived substances as pharmaceuticals in the 1940s through the 1980s. Importantly, the ease of standardization of single chemical entities into dosage forms, thereby allowing predictable outcomes of dosing, further supported the move to such pharmaceutical preparations. Even so, the importance of naturally derived substances for drug discovery and development was still strongly evidenced by the analysis published by Newman and Cragg in 2012 [5].

Interest in the study of plant-derived natural products is relegated to the academic community. However, the National Cancer Institute of the United States retained a core program of research on plant-derived natural products for the discovery and development of anti-cancer pharmaceuticals. The discovery and development of Taxol (Paclitaxel) in the decades of 1960s-1990s, as the most successful anti-cancer agent ever, caused a momentary resurgence of interest in natural products for drug discovery [6]. This interest has once more been replaced by a focus on new approaches to drug discovery such as combinational chemistry and computer-based molecular modeling coupled to high-throughput screening of bio-molecular models (receptors, enzymes, etc.) and most recently by our increased understanding of molecular genetics and immunology. The importance of plant-derived natural products within the pharmaceutical industry has waned significantly over the decades from the discovery of antibiotics and has practically disappeared today.

The emergence of resistance to single chemical entity agent materials whether a pharmaceutical (antibiotic-resistant infections, drug-resistant malaria, drug-resistant tuberculosis, numerous others), insecticide (insecticide-resistant mosquitoes and crop pests), or herbicide (herbicide-resistant weeds in our crops) has or is bringing the magic bullet paradigm into question [7]. There is an urgent need to identify novel active leads for the development of a new strategy to overcome the emergence of resistance. As was dramatically illustrated by the discovery of the "wonder" antibiotics of the 1940s and 1950s and the anticancer agent paclitaxel and others in the 1950s through the 1970s, nature is the prime source of such unique, novel lead discoveries. As emphasized by Dr. Norman Farnsworth, a leading world-renowned natural product researcher, "The World of Plants, and indeed all natural sources, represents a virtually untapped reservoir of novel drugs awaiting imaginative progressive companies [8]. The role of plant-derived natural products in the discovery of prototype pharmaceuticals arises from the observation that plants interact with their environment by chemical means. Plants protect themselves; attract pollinators, etc. by means of chemical substances. Those interactions are very effective. Since plants are rooted in place, they have evolved mechanisms to protect themselves by producing chemical defense substances.

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The function of these chemicals is to enhance the competitiveness of the plant in its environment, making it more successful, in turn creating the opportunity for it to propagate offspring. Because there are hundreds of thousands of plant species all producing large numbers of discrete chemical defense substances and chemical signaling for mutual cooperativity among neighboring organisms, thus there are literally millions of chemical structure types to select from for evaluation for pharmaceutical application. It may also be argued that these substances represent "nature's combinational chemicals" and that they have the advantage of already having been screened evolutionarily for pharmacological utility. The following three compounds are recent important examples of plant-derived natural products which have defined new pharmaceuticals. Paclitaxel, a diterpene isolated from the stem bark of the Western Yew, *Taxus brevifolia,* is the most significant anticancer agent developed in the last several decades. It is unique among currently available antitumor agents in that it enhances tubulin polymerization, acting via that mechanism as a mitotic poison. Topotecan, a more recently approved anticancer agent is a derivative of the natural product camptothecin, an alkaloid isolated from *Camptotheca accuminata.* It also has a unique mechanism of action; functioning as an inhibitor of topoisomerase I.

Literature Review

Drug-resistant strains of malaria claim hundreds of thousands of lives worldwide each year. Significant effort continues for the development of new, safer agents effective against drug-resistant malaria. For this, researchers have turned once again to a plant-derived natural product. For centuries, extracts of the plant known as Qinghao *(Artemesia annua)* were used in Chinese Traditional Medicine for the treatment of malaria, including cerebral malaria. Chinese investigators reported the isolation and identification of the active constituent of Qinghao as the unusual sesquiterpene endoperoxide, artemisinin. Semisynthetic derivatives of artemisinin have now been approved in several countries and represent current drugs of choice for treatment of chloroquine-resistant malaria. Unfortunately, as might be expected, we are seeing the development of resistance to these important agents. An important element of the development of resistance is the reliance on single chemical entity agents. Nature is communicating a strong message to us when we examine the composition of natural product preparations. These are mixtures of many compounds, often closely related to what we perceive as the "active principal".

The accepted role of these natural products as protective from pathogens and consumers and the lack of resistance development by the target organisms should clue us that mixtures make an important contribution to prevent resistance development. Numerous observations supporting the role of mixtures to suppress or prevent the development of resistance have been published. As long ago as 1976, Pimentel and Bellotti published a study reporting prevention of resistance development to a mixture of toxic chemicals to house flies raised in a culture (Figure 1). More recently, Zhao, et al. published the observation that incorporating more than a single form of B. thuringiensis toxin into the genome of a crop plant suppressed resistance development (Figure 2) [9].

Evaluation of a natural product insecticide, Azadirachtin, as a single agent versus an extract of its source material with an equal concentration of "active" similarly showed the extract mixture suppressed resistance development (Figure 3). The discovery and development of artemisinin for treatment of chloroquine-resistant malaria was heralded as a breakthrough and the discoverer was awarded the 2015 Nobel in

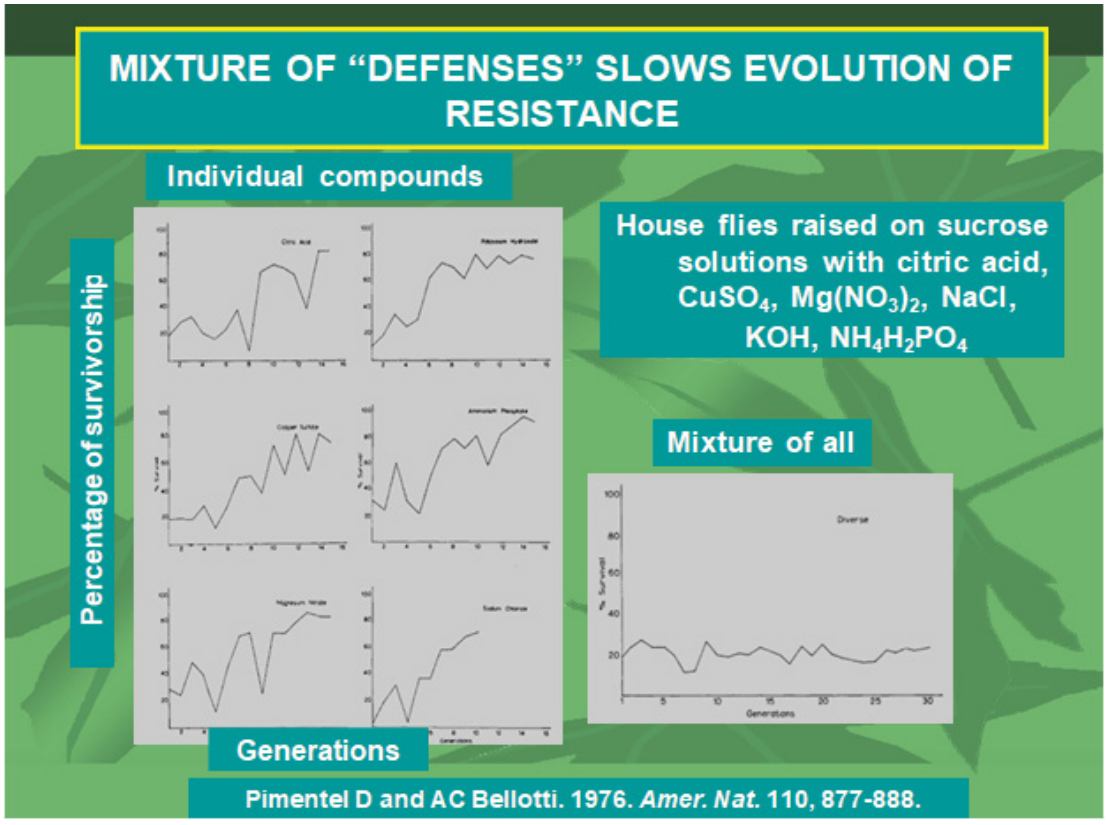

Figure 1: A combination of individual toxic substances suppresses resistance development.

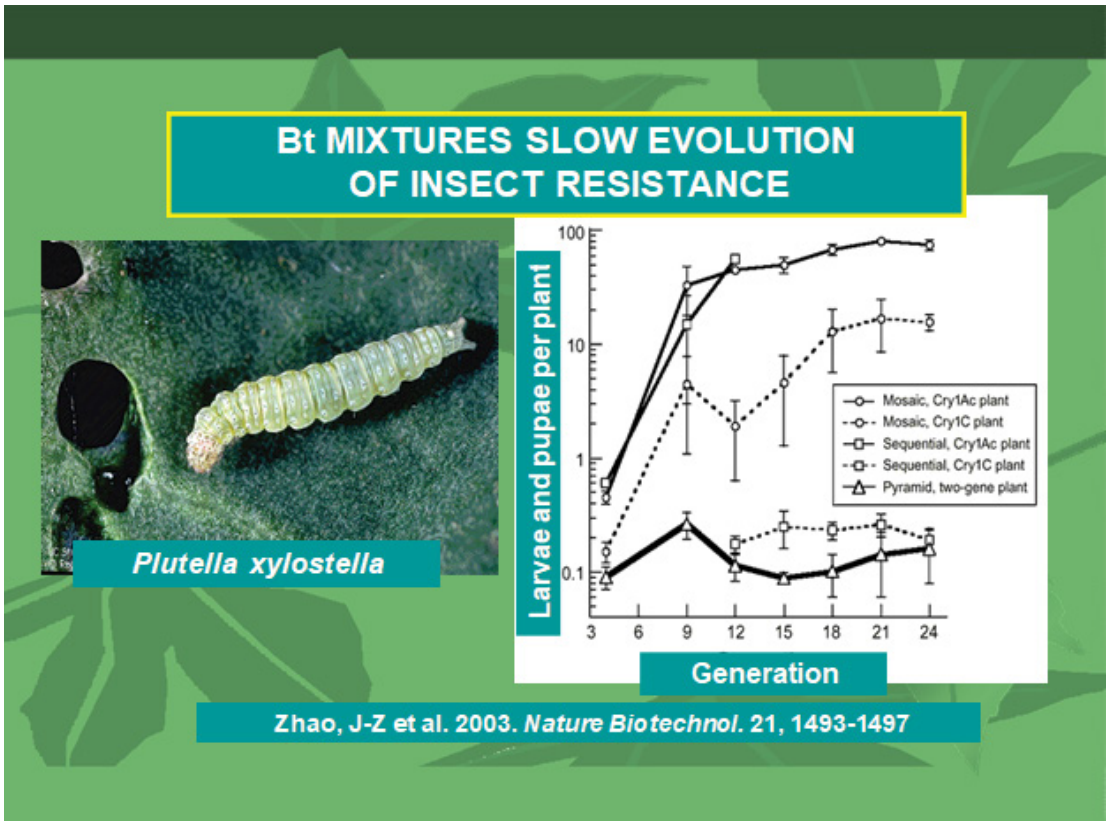

Figure 2: Co-expreesion of Bt toxins suppresses resistance development.

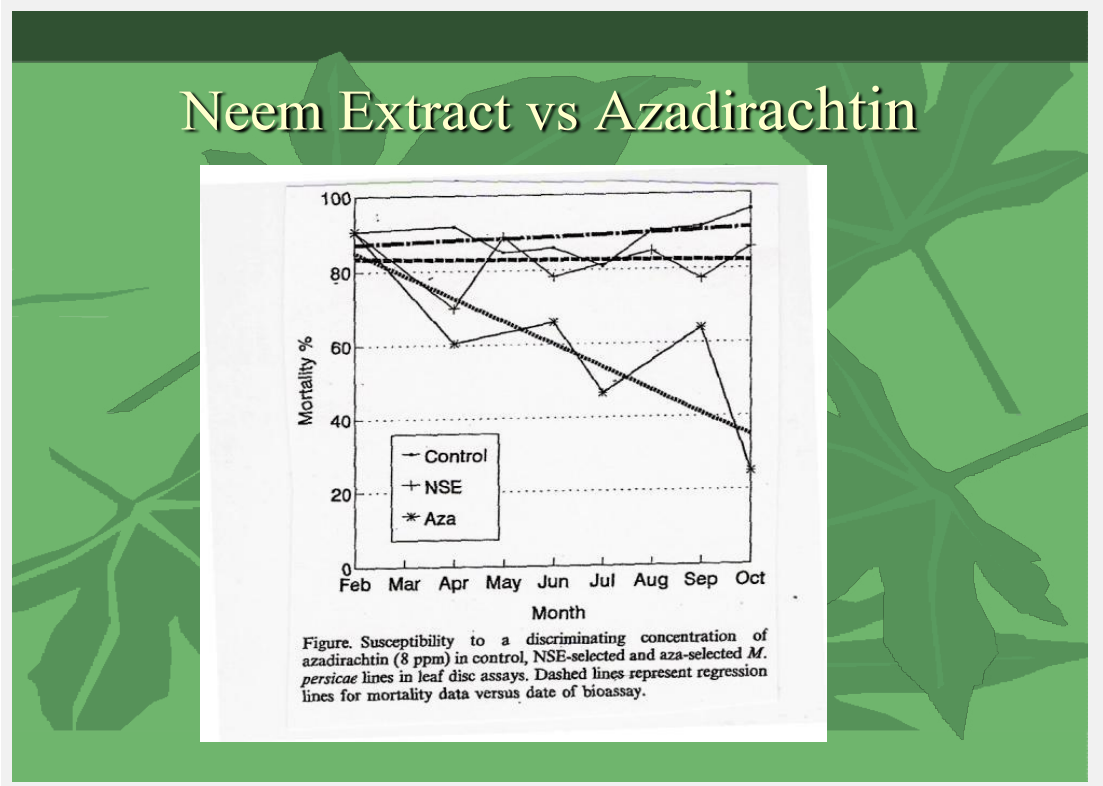

Figure 3: Neem extract with its complex mixture of substances suppresses resistance development.

Medicine. However, broad-scale use of artemisinin has led to the emergence of resistance even when the WHO has recommended the combination with other antimalarials. Recently the laboratory of Dr. Pamela Weathers has published work demonstrating the efficacy of an intact Artemisia leaf preparation to cure patients who had failed two courses of standard artemisinin therapy with the recommended WHO combination drugs [10-12].

In a recent series of publications, Chinese researchers have been comparing pharmacokinetic outcomes between single-isolated active-principal preparations and traditionally utilized whole-plant extracts containing the active principal in equivalent concentration are orally administered to laboratory animals, rodents [13].

They demonstrated a consistently higher bioavailability and interestingly when examining the organ tissue distribution of paclitaxel, a potent cancer chemotherapeutic agent, they found that the extract not only significantly increased oral bioavailability, as much as 10-fold, but also increased the distribution of paclitaxel to all organs examined [13]. How can/might the mixture of compounds present in the intact leaf material or whole plant extract influence the efficacy of the preparation? There are numerous possibilities- compounds present may add to or synergize the activity of the "active principal"; those compounds may enhance absorption, distribution, or modify the metabolism of the active principal. Those compounds may serve to suppress resistant mechanisms of the infectious agent or those of undesired/unwanted species. There may well be additional mechanisms of enhancing efficacy as yet unidentified. This observed enhancement of efficacy has been dubbed "the entourage" effect [14].

In the case of cytotoxic chemotherapeutic agents, greater systemic distribution, either from greater bioavailability (blood level) or suppression of tissue-protective efflux mechanisms (ABC transporters), potentially could lead to greater efficacy but also raise the risk of greater side effect toxicities, a major limiting factor of cancer chemotherapy. The public interest in plant-based preparations (Botanical Drugs, even botanicals popular as dietary supplements ("nutraceuticals") and the movement to legalize marijuana (medical marijuana) sweeping the country strongly supports that we revisit the development of plant-based drug preparations.

Botanicals: A "new" class of drugs

The guidance provided by the FDA defines Botanical Drugs as a product that contains as ingredients vegetable materials, which may include plant materials, algae, macroscopic fungi, or combinations thereof, that is *used as a drug*. (Note: NB: "Botanical" ingredients are not restricted to the categories of "drugs" or "dietary supplements" [15]). Botanicals are--and have been-- cosmetics, foods, food ingredients, as well as dietary ingredients. They may also be a medical device (e.g., dental alginates), or biologic (allergenic vaccines, etc.). Based on what the product sponsor wants to do with the botanical ingredient, botanicals are drugs in the United States when the product meets the legal definition of a drug. Most botanicals (with the exception of perhaps, psyllium, and a very limited number of other ingredients), would be "new" drugs, as they are not already recognized as Generally Recognized as Safe for intended use in a drug in the US. This means that an ingredient that is determined to be a "GRAS ingredient intended to be used in food" (as would be the case for "GRAS" (Generally Recognized as Safe) botanicals for dietary supplements) would not qualify as "GRAS intended for use in drugs". As noted below as "new" drugs, the overwhelming majority of botanicals would need to be developed via the Investigational New Drug (IND)/New Drug Application (NDA) process The Botanical Drug may be available/formulated as (but not limited to) a solution (tea, e.g.), powder, tablet, capsule, elixir, topical, or injectable. Specifically excluded are fermentation products, highly purified [or chemically modified] botanical substances, genetically modified plants, allergenic extracts, and vaccines which contain botanical ingredients.

Clinical development of botanicals as new drugs

A more extensive and detailed discussion of the process of clinical development of a Botanical Drug may be found in Dou et al. [16]. A botanical raw material (i.e., plant part(s) from a single plant species or an extract including those partially purified but still containing one or more classes of molecules) can be studied as a botanical drug product. A sponsor need not establish the clinical effects of each molecular entity in a botanical product derived from a single part of a plant, as would be required for a fixed combination of several compounds. FDA recently modified the fixed-combination drug regulation to potentially exempt certain complex botanicals, containing many distinct components derived from various botanical raw materials, from the requirement to demonstrate the contribution of each botanical raw material to the whole combination botanical drug. With many components (e.g., 4-5 or more), the combinatorics make factorial studies to evaluate the contribution of the main effects and their interactions (e.g., ABCD>ABC, ABD, BCD, ACD…) simply infeasible.

The need for an IND to conduct human clinical research of botanicals

A botanical product can be regulated as a drug or a dietary supplement based on its intended use and how the product is labeled. In general, whether an investigational new drug application (IND) is required for human research involving a botanical marketed as food/dietary supplement depends on whether the intended use is for a structure/function claim (no IND needed) or a disease claim (IND needed), and not on the physical or chemical properties of the product. Regarding studies evaluating certain cellular mechanisms or pharmacodynamic responses, such as antioxidant activity, immune modulation, and COX-2 inhibition, whether an IND is required could be based on whether the clinical data will be used to support the drug's future labeling and marketed use, and on whether there is a safety concern. For example, a clinical investigation designed to study the relationship between a dietary supplement's effect on normal structure or function in humans (e.g., guarana, the seeds of *Paullinia cupana* Kunth, and maximal oxygen uptake) or to characterize the mechanism by which a dietary supplement acts to maintain such structure or function (e.g., fiber and bowel regularity) would not need to be conducted under an IND. [For inquiries on whether an IND will be necessary for certain human studies of herbal products currently marketed or intended to be used as food, dietary supplements, and cosmetics, researchers are advised to contact the FDA (INDsFoodsDietarySuppCosmetics@fda.hhs.gov)]. An IND will be required for a botanical product if it will be used as a drug to treat, mitigate or prevent a disease or its related conditions in the proposed clinical study. Review of studies conducted under IND allows the FDA to help ensure that the research is well designed and is attentive to safety concerns. Especially for large and costly trials with important impacts on patient management; advice from the FDA can help ensure that the clinical data generated will be useful in supporting a proposed use or demonstrating an effect.

Basic principles of the botanical guidance supporting early phase trials

The basic requirements for initial botanical drug investigations take into account the unique features of botanicals and the practical challenges in their development (e.g., complex mixtures in which the

active ingredient is unknown or difficult to quantify, and substantial prior human experience exists). In principle, the standards for product quality and the evidence of effectiveness and safety that are required for all new drugs approved by FDA also apply to new botanical products intended to be marketed as drugs in the U.S. These standards can be relatively straightforward for demonstration of effectiveness and safety, but product quality assurance needs to take into account the fact that botanicals are mixtures in which the active compounds may not be known. The regulatory intent is not to create a separate category of therapeutic agents for botanicals, but to ensure the same degree of confidence in their quality and clinical usefulness as exists for non-botanical drugs.

The first trial under an IND

Optional phase 1 studies: Unlike investigations of small molecules without previous human experience, investigations of botanical drugs have more flexibility in the timing or sequencing of phase 1 and 2 trials in a development program. For many marketed botanicals with extensive human experience, the typical phase 1 study for new molecular entities may not be necessary. Therefore, many IND sponsors could skip phase 1 trials and initiate phase 2 controlled trials in patients to seek preliminary evidence of the efficacy of the botanical drug candidate, usually at the doses suggested by the existing human experience in a dietary supplement or herbal medicine applications. A randomized, parallel, fixed-ratio of multiple botanicals (also referred to as fixed-dose combinations) if desired, and dose-response study may be particularly useful as an initial trial for botanicals.

Initiation of phase 2 studies: Analysis of previous human experience with products used as herbal medicines or dietary supplements, including case reports, other non-controlled historical data, and preliminary trials reported in the literature, may provide useful information to support the safety of initiating phase 2 trials under an IND without prior formal phase 1 trials. Previous experience may also provide information regarding specific disease indications, dose determination and rationale for controlled studies under INDs. If the purpose of the later clinical studies is to demonstrate the clinical benefit of a botanical product that was suggested by the phase 2 study, it is clear that the composition of the product (e.g., the number of herbs/botanical raw materials and weight of each herb) and the process used to prepare the botanical product (e.g., solvent to herb ratio if extraction is involved) will need to be determined and controlled appropriately prior to conducting the later-phase-controlled trials. A marketed dietary supplement having adequate safety information may enter phase 2 trials. Generally, however, larger amounts of products in multiple and reasonably consistent batches will be needed for the phase 3 trials. Researchers choosing to study a marketed over-the-counter botanical product often face a challenging task of selecting the right product, especially when several similar products are available. The dietary supplement/herbal medicine manufacturers may not agree or be able to provide a reasonably well-characterized product and related quality and process information to the sponsor and provide reference data for an IND submission. As quality-related variables and many other factors may potentially alter clinical results, promising results from a phase 2 trial should be corroborated by other studies. If biological assays are available, they could aid in dose selection and batch-to-batch consistency evaluation early on. With respect to serious illnesses for which there is an established effective therapy, sponsors of botanical drugs are encouraged to use an "add-on" design for the initial trials. That is, the botanical drug would be compared to a placebo, each being added to the standard of care; this creates a three arm study; placebo, placebo plus standard of care, and placebo plus botanical drug candidate.

The efficacy of the botanical drug can then be assessed as equivalent or superior to the current standard of care. However, when an investigational new botanical drug is tested together with approved drugs in an "add-on design" or used as part of a combination with other approved drugs, the possibility of unintended interactions needs to be ruled out by conducting drug-drug interaction screening studies. If indicated, botanical drug interaction studies in healthy volunteers at appropriate doses and dosing intervals could provide additional information to support controlled studies in patients [17].

Chemistry, Manufacturing and Controls (CMC) challenges

Previous human experience with a botanical may support initiation of clinical studies of the botanical product by a sponsor providing significantly less CMC, nonclinical pharmacology, and toxicology data than required for studies compared to a synthetic drug with no prior human exposure. For example, sponsors are not required to further purify or to identify the active ingredients of complex botanical extracts that are likely to contain multiple classes of molecules. However, unlike pure non-botanical drugs, typical CMC controls of drug substance and drug product may not be sufficient for a botanical product. Additional botanical raw material controls (including standardized good agricultural and collection practice) are likely to be required to ensure batch-to-batch consistency during late-phase-drug development. As outlined in the Guidance, additional methods are recommended to ensure batch-to-batch and lot-to-lot consistency of the botanical drug. (Besides the statistical methods outlined here for chemical characterization of the product, stringent raw material control, process control, a relevant bioassay and prehaos other means, a "totality of evidence".

Advancing a botanical into late phase clinical development

In general, trial designs for naturally-derived complex drugs are not appreciably different from designs used for small molecules, which are usually homogeneous and highly purified. One of the most important issues for the phase 3 trial is the determination of the sample size based on estimates of efficacy over the control group derived from prior studies. Sponsors are cautioned not to interpret the phase 2 data or uncontrolled data overoptimistically, and thus leaving the phase 3 trials too small to adequately evaluate the treatment effect of the investigational drug. Studying complex-naturally derived mixtures, such as botanicals and their combinations, does have unique challenges in terms of reproducibility or consistency, especially when the active ingredients at the molecular level are not fully characterized or quantified. To address this concern, representative drug substance/product batches, which have "acceptable levels" of chemical heterogeneity, should be selected for study in phase 3 trials. Inclusion of multiple doses, such as the approach adopted in the phase 3 trials of Veregen® (10% and 15% ointment) and Fulyzaq® or Mytesi® (125 mg, 250 mg, 500 mg BID Bi-ingestions daily), not only demonstrated that the drugs are safe and effective, but also that the small uncontrollable quality variations that existed in the botanical products would not affect the clinical outcomes with the drugs.

Demonstration of dose-response effect to support drug approval

Clinical response data for a botanical drug may not only demonstrate that the studied doses are more effective than placebo or active control (or not inferior to active treatment), but may also indicate that the effect of the drug on clinical outcomes is not sensitive to dose. Thus, dose response can be used to indicate whether the treatment effect is potentially affected by variations of different batches

for a botanical drug. If a randomized, multiple-dose, parallel-group design, phase 3 study demonstrates a similar treatment effect across multiple doses, concerns about the impact of variability in chemical composition across batches may be mitigated. For example, there was no significant difference in the clinical response to Veregen® between the two doses tested (10% and 15%) with both doses showing significant treatment effects for genital and perianal warts over the vehicle control groups. The essentially flat-dose response curve indicated that certain natural variations and the residual uncertainties of the drug substance composition would not be expected to be critical to therapeutic effect. In the case of Fulyzaq®, indicated for the treatment of HIV/AIDS-related diarrhea, the decision to approve was based on the overall findings of safety and effectiveness from a total of 696 HIV-positive patients who received Fulyzaq® at dose ranges between 125-500 mg, bid, in three placebo-controlled trials.

A significantly larger proportion of patients in the Fulyzaq® 125 mg twice daily group experienced clinical response (≤ 2 watery stools per week) compared with patients in the placebo group (17.6% vs. 8.0%, 1–sided $p < 0.01$); this response was similar to that for the 500 mg, BID dose group. When administered at 125 mg BID, the estimated gastrointestinal lumen concentration of crofelemer (the drug substance) was 240 μM, which was many folds higher than the *in vitro* concentration required for inhibition of chloride secretion, the drug's known mechanism of action. Pharmacology studies also suggested that Fulyzaq® at 125-500 mg BID could essentially saturate the chloride channels; thus, no clear dose response was observed or expected in this dose range. Taken together, the multiple-dose phase 3 trials and the pharmacology studies suggested that drug saturation at the sites of action and clinical response were not likely to be affected by the chemical variations in the drug batches used during phase 3 trials. The drug's known mechanism of action also made it possible to implement bioassays to help ensure the batch- to-batch consistency of Fulyzaq®.

Multiple-batch Phase 3 clinical trials of botanical drugs to demonstrate consistency

As, it is often not practical to identify all the active components in a botanical drug at the molecular level, the entire component mixture of the botanical drug substance is generally considered as the active pharmaceutical ingredient. Composition variations in the botanical raw materials are expected, and botanical drug substances are also expected to have variations in their chemical composition, including those arising from purification procedures, such as standard Ginkgo extracts, green tea extracts, and so on. For, highly purified homogeneous small-molecule drugs, the clinical effects can be linked to the active pharmaceutical ingredient when the impurities are adequately controlled. However, for complex botanical preparations, data from multiple-batch and multiple-dose clinical trials are needed to better ensure batch-to-batch consistency in terms of both quality and therapeutic effects. When, Conventional Chemistry, Manufacturing and Control (CMC) mechanisms, like those routinely applied to small molecule drugs, are viewed as inadequate to ensure quality and therapeutic consistency of heterogeneous botanical and other naturally- derived complex drugs, how a sponsor selects representative batches for its confirmatory trials is an important consideration. The need for multiple-batch and multiple-dose studies will be critical to assure consistent clinical benefit to patients should the botanical preparation be approved as a Botanical Drug. The quality data for the multiple batches used in phase 3 trials of a botanical drug could provide important data for establishing specifications for the drug once approved. For example, the drug substance of Veregen®, sinecatechins (aka Polyphenon E®) was defined to constitute 85% to 95% (by weight) of catechins, which includes more than 55% of Epigallocatechin Gallate (EGCG) and seven other catechin derivatives.

The specifications of total catechins and the individual catechins in the drug substance were established based on the analytical data of the clinical batches. The batches chosen for phase 3 trials should be representative of the proposed marketing batches and should not be so homogenous that post-approval large scale production of the drug would be impractical. Using literature data for the standardized green tea extract, Polyphenon E®, as a hypothetical example, the usefulness of selecting multiple batches for clinical studies is outlined. One batch of Polyphenon E® was reported in the literature to contain 65% of (-)-EGCG and 89.5% of total catechins (Rizzi et al.). Another batch of Polyphenon E® used in a chemoprevention animal study was very similar in EGCG (65%) and total catechins (89.4%) contents [18]. Those two batches were only slightly different in the percentages of several minor catechins. A third batch of Polyphenon E® used in an early study contained a much lower percentage of EGCG (51.4%) [19].

If Polyphenon E® batches closely resembling the first two very similar batches were selected for phase 3 trials, then the EGCG and catechin specifications for future marketing batches would probably also be very tight. If batches of Polyphenon E® with wider percentage ranges of EGCG (e.g., 51% and 65%) and catechins were included in the trial and considered in the determination of specifications, and if no relationship was observed between the content of the batches and the clinical effect, then the specifications for EGCG/catechins post-approval could be wider. When a relatively large number of batches of the drug will be studied in phase 3 trials, it is recommended that sponsors analyze batch effects on clinical endpoints (i.e., batch effect analyses) to rule out any effect of known variables (e.g., batches made from raw botanical materials collected from different region/sources) and observed compositional variations (e.g., the specified weight % ranges of known marker compounds, such as EGCG and other catechins in Polyphenon E®) on clinical outcomes.

The goal of batch analyses is to identify and quantify the potential effects of chemical heterogeneity on clinical outcomes for subjects who receive different batches in the study. Despite the importance of batch-effect analyses that help ensure batch-to-batch consistency of effects, these analyses are usually considered exploratory, with no formal requirement of control of the Type I error rate (i.e., the false positive rate). Randomization of subjects to different batches in each site will facilitate batch effect analyses. If formal batch effect analyses are warranted (e.g., use of a relatively large number of phase 3 batches with apparent heterogeneity among those batches), it is important for the sponsor to design clinical studies to facilitate these analyses and to pre-specify in the statistical analysis plans how these analyses will be carried out.

Safety assessment of botanical products during clinical development

For botanicals as well as for synthetic or highly purified drugs, absolute safety does not exist, and the FDA must assess risks considering clinical benefits as per Code of Federal Regulations (CFR) Title 21, Part 312.22 [20]. As is the case for synthetic or highly purified drugs, the best safety data on newly developed botanicals will be derived from controlled trials, but for chronic indications, long-term, open-label extensions also will be important. For chronic conditions, exposures of at least 6-12 months' duration are usually appropriate as per ICH guidance, E1A, the extent of population exposure to assess clinical safety for drugs intended for long-term treatment of non-life-threatening conditions in March 1995 [21].

Botanicals as fixed-combination drugs

The FDA Guidance on Botanical Drugs indicates that a botanical drug product derived from a single part of a single plant species (i.e., a single herb) is not deemed a combination drug for which the contribution of each component to the treatment effect must be demonstrated. Even for multiple-herb combinations, if there are no safety concerns, early phase trials can be initiated without addressing the contribution of each herb to the drug's effect. The fixed- combination drug regulation has been revised in a proposed rule to give more flexibility to the evaluation of complex mixtures, like botanical combinations used in traditional medicine systems (e.g., Ayurveda, traditional Chinese medicine). Manufacturers of multiple-herb formulae may not be required to demonstrate in a marketing application that each herb contributed to the final product if it is not practical to do so (e.g., a factorial study to test 4-5 or more components is usually not possible). This change should encourage further clinical development of herbal medicines, which often contain several herbs used together, as new botanical drugs. Thus, when it is impractical to study the treatment effect of a complex multiple-herb combination along with each herb in the combination product, the focus should be on the clinical development of the combination rather than its components.

Potential botanical drug products from *Cannabis*

Cannabis-based medicines are and are validating the whole-plant, or full-spectrum extract, as the medicine of choice versus single compound cannabinoid isolates. Single cannabinoid medicines like GW Pharma Epidiolex® (a single component CBD preparation developed under the current FDA paradigm of pharmaceutical development) and other CBD only plant medicines have approximately only 40% efficacy to halt epileptic seizures in children, while a study found that families that moved to Colorado for *Cannabis* treatment achieved 60% success, possibly due to the use of preparations containing additional *Cannabis* components, such as low-levels of Tetrahydrocannabinol (THC) and other terpenes found in *Cannabis-based* CBD products. A meta-analysis of published reports by a Brazilian group suggests that the whole plant preparations were as much as 5 times more efficacious than products of CBD only [22]. *Cannabis* evolved as an illegal medicine, with many of the early studies using plants or raw extracts identified only by the name of the plant as a select cultivar (variety). With limited access to process equipment and analytical laboratories, many of these early products had no other means of characterization. This early characterization of medicines by strain led to researchers trying to correlate medical outcomes by strain, as was done in the past. *Cannabis* strains are also named as chemovars, which often have significant differences in their chemical profiles, such as concentrations/ratios of CBD, THC, and other cannabinoids and minor terpene components. Other components in *Cannabis* include flavonoids, essential oils, terpenoids, among other things. With both THC and CBD as approved new drugs and diversities in their chemical profiles, *Cannabis chemovars* are excellent candidates for the development of new Botanical Drugs. One such example is GW Pharma's Sativex, a *Cannabis* botanical drug which contains THC and CBD at approximately 1:1 ratio and approved for multiple sclerosis in more than 25 countries [23]. However, attempts to study Sativex in the United States for pain management in advanced cancer patients failed to produce the desired clinical benefits. Various other *Cannabis* products derived from high-THC or high-CBD chemovars are also suitable candidates for botanical drug development. One potential benefit for developers of botanical drugs, including those vast numbers of *Cannabis*-based products, is that a generic copy of the botanical drug could not be easily made. It could be very challenging to demonstrate the sameness between two batches of a botanical product, let alone between two *Cannabis* products, especially if much of the CMC information are usually trade secrets or otherwise not in the public domain.

Facing the challenges in chemistry, manufacturing, and control for botanical drugs

To progress to successful Botanical Drug Development, we must develop means to assure the botanical preparation can be predicted to provide the desired efficacious medical outcome. In the current pharma paradigm, this goal is ultimately accomplished by strict control of the manufacturing process and analysis of drug dosage forms with characterization, standardization, and dosing defined around one chemical or a limited number of components. Chemical characterization is a broad term, encompassing analytical chemistry technologies and instrumentation. Single compound products (today's standard drugs) are easily measured by targeted analysis: foreknowledge of the active pharmaceutical ingredient enables the analytical method to identify and quantify the desired, or targeted, compound. Targeted analyses are used extensively to identify and quantify known sets of compounds. For instance, THC and CBD content in *Cannabis* are important for regulatory compliance, to measure quality and to standardize dosing, so methods are and have been developed to target these compounds for accurate quantitation.

One issue with targeted analysis of a complex botanical drug is that it is myopic and limited by current knowledge. When a new active compound or component is discovered the old database is obsolete. Targeted compound analyses and resulting correlations may not define causal relationships because of the influence of additional "hidden" components that affect the activity of the targets and are not included in the targeted analyses. Non-targeted analyses attempt to measure the chemical composition of the plant material using spectral methods such as NIR, UV-vis, NMR, or MS. Spectral methods offer the advantages of providing rich information with a fast analysis, less sample preparation, and fewer consumables and waste compared with other analytical techniques, such as chromatography. Chemical profiling (aka chemotyping) using spectral methods focuses on the shape of the complex spectrum (i.e., the distribution of features) that may be correlated to the medicinal property, not a particular compound of interest. Utilizing powerful statistical techniques, non-targeted analyses can look for patterns, changes, and correlations within mixtures before the compounds are identified. A chemical profiling approach can locate compounds that correlate with properties of interest, then these compounds are identified, and targeted methods of analysis are developed for study and dosing.

The conventional approach would be to attempt to identify and isolate all compounds and then test them individually for their activity, which for botanical materials is just not feasible. Non-targeted chemical fingerprinting is already used in the dietary supplements industry-botanical raw material ingredients are characterized initially by sophisticated "gold standard" methods, only to have acceptance of incoming raw materials approved by NIR spectral fingerprints to detect deviations in the source and product streams that arise from adulteration, mislabeling, and decomposition. Recent work for chemical profiling *Cannabis* using a UV microplate reader that can rapidly collect spectra of extracts in a 96-well plate has been published by Chen et al. [24]. Another study used nano-electrospray high-resolution mass spectrometry for characterizing *Cannabis* samples [25]. This study used an experimental design approach to optimize mass spectral preprocessing. A non-targeted approach for studying

the metabolic phenols from cranberry by fuzzy chromatography-mass spectrometry used an expert system combined with bioinformatics [26].

Wang et al. devised a rapid method for extracting and chemotyping *Cannabis* using NMR spectroscopy [27,28]. She also studied the effect of preprocessing on the direct infusion of *Cannabis* extracts into a high-resolution mass spectrometer [29]. Typically, there are two general approaches to chemometric chemical profiling. The first is the modeling approach which is sometimes referred to as one-class classification. The second is classification where the spectra will be assigned to one of multiple classes. Modeling approaches work by finding similarities in the spectral shapes and are generally not as selective as the classification approach. Often modeling is used for untargeted or exploratory analysis.

Classification relies on finding differences in the spectral shapes of spectra that belong to different classes. Because it is using differences, these approaches are more selective and have greater discriminatory power. Classification usually is used for targeted approaches, for example, classes of spectra may be classified as pharmacologically active or inactive. One would need a group of spectra from both the active and inactive products. For modeling, a set of representative spectra is collected, and a model is built. The simplest perhaps is the calculation of the average spectrum. Then, a boundary is placed around the model and in the simplest case; it could be defined as the distance of the spectrum furthest away from the average. Then when a new spectrum is presented that is outside the bound, it would be rejected from the model and for the case that it is within the bound it would be accepted. Basically, all models rely on this approach albeit the model may be much more complicated than using an average spectrum. Soft independent modeling of class analogies (SIMCA) is the most popular and its model is a basis of principal components [30].

These approaches have been used for years by industrial chemical engineers for monitoring large-scale process streams [31,32]. Other methods that may be used are cluster analysis or support vector machines. For classification, multiple sets of representative spectra are collected with each set representing a class. The classification methods then try to optimize a model that enhances the difference among the classes. Perhaps, the oldest classification method is Linear Discriminant Analysis (LDA), which by the way was developed for botanical classification. Unlike modeling, there are many classification methods. One popular method is partial least squares discriminant analysis (PLS-DA). Other trendy methods are Support Vector Classifiers (SVC) and Support Vector Machine Trees (SVM-Tree) that are easy to use and parameter-free. A word of caution, classification methods are very powerful so the models can over fit the spectra (i.e., use noise and baseline artifacts in the spectra) so they must always be validated with an external prediction set (i.e., one that was never used in optimizing or building the model). A problem with classification methods is that sometimes when a new spectrum is presented that does not correspond to any of the classes, it will be misclassified. This problem has been solved by Chen and Harrington who have combined modeling and classification methods into a pipeline. At the beginning of the pipeline, a modeling method is used to determine whether a spectrum is consistent with previously collected spectra. If not it is rejected from the pipeline as an outlier, if it is consistent then it will be classified into one of the target classes by using a subsequent classification method. Modern instrumentation and advanced statistical techniques enable the study of the human genome and correlate that to complex botanical drugs.

Artificial intelligence can glean information from nuances in the data that escape the human eye. Such studies are important because synergistic effects between compounds in botanical mixtures are well documented, even though there has been little hard research to demonstrate the entourage effect. Most research has been focused on the concept of the "magic bullet", i.e., the active principal being responsible for the observed biological activity. As more botanicals are evaluated, we will likely, almost certainly, see the entourage effect as the rule, not the exception. This realization will undoubtedly provide for more efficacious medicines in the future and also help slowly or even preclude resistance development, a strong argument to develop botanical drugs. Metabolomics studies are also facilitating the development of new botanicals since such studies allow one to examine the influence of environmental, seasonal, and other factors on the production of plant metabolites. In the case of *Tithonia diversifolia,* a metabolomics study revealed the influence of environment on its metabolic profile, in which the variation in the production of phenylpropanoids and sesquiterpene lactones in the plant appeared to be a direct response to changes in conditions of its surrounding environment [33].

On the other hand, metabolic studies on *Copaifera langsdorffii* specimens, grown from seeds collected from 10 different regions in southeast Brazil, and grown in the same field, revealed that the overall effect of environmental factors on the production of phenolic metabolites was uniform among *C. langsdorffii groups* [34]. Metabolomic studies constitute a key tool to understand the factors that affect metabolite production in medical plant species and contribute to the standardization of raw material for the production of botanicals.

Discussion and Conclusion

Complex botanical derived products, including those used in or marked as traditional medicines, could still be viable candidates for botanical drug development. FDA's approvals of Veregen and Fulyzaq set up good examples for the industry to learn from. Challenging CMC issues may be adequately addressed by controlling the source and quality of the botanical raw materials fixed manufacturing processes, extensive chemical analyses, as well as biological assays to ensure batch-to-batch consistency. Synergism, as the examples demonstrated in the earlier parts of this paper, could be an important indicator for selecting botanical drug candidates. Multiple molecules from one botanical extract or combinations of multiple herbs, should they possess additive or synergistic effects, may be further developed with better potential as new drugs. A documented history of human data could allow a sponsor to proceed directly into a clinical evaluation of efficacy (and safety) thereby bypassing potentially long and expensive preclinical development efforts. Early clinical evidence of efficacy also reduces greatly the risk of development failure, a major common issue in single new chemical entity drug development.

We recognized that there have been inadequate funding available for natural products in general and botanical drug development in particular. Developing a drug to US drug standards indeed takes 'real money', but unfortunately most investors still view botanicals in the US as "foods" or "supplements", i.e., there is not sufficient profit margin there to warrant the investment. To change this view, an investor must be confident that the product that they invest in has proprietary insulation, and that both current and future regulation of the product will protect the product -- as a "drug". Also, the indication (expected medical application) must represent a sufficiently large market to produce a significant return or an orphan drug with highly profitable price. The currently approved US botanical drugs have not fully met that hurdle of high profitability. However, no generic competitions long after their exclusivity expired may help investigators and investors

to realize that challenges in CMC (including complex compositions) could actually serve as "trade secrets" and protect the innovators of the approved botanical drugs in a similar way as patents for purified small molecule drugs. There are numerous unmet medical needs for which botanical drugs could provide real patient benefit and real monetary return to investment.

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References

1. Schwartz RS (2004) Paul Ehrlich's magic bullets. New Engl J Med 350: 1079-1080.
2. Strebhardt K, Ullrich A (2008) Paul Ehrlich's magic bullet concept: 100 years of progress. Nature Reviews Cancer 8: 473-480.
3. Aminov RI (2010) A brief history of the antibiotic era: Lessons learned and challenges for the future. Front Microbiol 1: 134.
4. Bosch F, Rosich L (2008) The contributions of Paul Ehrlich to pharmacology: A Tribute on the Occasion of the Centenary of His Nobel Prize. Pharmacology 82: 171-179.
5. Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Natu Prod 75: 311-335.
6. Wani MC, Horwitz SB (2014) Nature as a remarkable chemist: A personal story of the discovery and development of Taxol®. Anticancer Drugs 25: 482-487.
7. Ventola CL (2015) The antibiotic resistance crisis: Part 1: Causes and threats. Pharmacy and Therapeutics 40: 277-283.
8. Brahmachari G (2008) Mother nature - An inexhaustible source of drugs and lead molecules. Natural Products: Chemistry, Biochemistry and Pharmacology, Alpha Science International Ltd, Oxford, UK.
9. Zhao JZ, Cao J, Li Y, Collins HL, Roush RT, et al. (2003) Transgenic plants expressing two Bacillus thuringiensis toxins delay insect resistance evolution. Nat Biotechnol 21: 1493-1497.
10. Daddy NB, Kalisya LM, Bagire PG, Watt RL, Towler MJ, et al. (2017) Artemisia annua dried leaf tablets treated malaria resistant to ACT and i.v. artesunate: Case reports. Phytomedicine 32: 37-40.
11. Desrosiers MR, Weathers PJ (2018) Artemisinin permeability via Caco-2 cells increases after simulated digestion of Artemisia annua leaves. J Ethnopharmacol 210: 254-259.
12. Munyangi J, Cornet-Vernet L, Idumbo M, Lu C, Lutgen P, et al. (2019) Artemisia annual and Artemisia afra tea infusions vs. artesunate-amodiaquine (ASAQ) in treating Plasmodium falciparum malaria in a large scale, double blind, randomized clinical trial. Phytomedicine 57: 49-56.
13. Zhang XL, Lv JJ, Wang LL, Shao H (2016) Comparison of pharmacokinetics and biodistribution of 10-deacetylbaccatin III after oral administration as pure compound or in Taxus chinensis extract: A pilot study. Planta Medica 82: 230-237.
14. Ben-Shabat S, Fride E, Sheskin T, Tamiri T, Rhee MH, et al. (1998) An entourage effect: Inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur J Pharmacol 353: 23-31.
15. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/botanical-drug-development-guidance-industry
16. Dou J, Beitz J, Temple R (2019) Complex drugs, springer development of plant-derived mixtures as botanical drugs: Clinical considerations. (1st edn), The Science and Regulations of Naturally Derived Nature, Heidelberg, DE.
17. Huang SM, Temple R, Throckmorton DC, Lesko LJ (2007) Drug interaction studies: study design, data analysis, and implications for dosing and labeling. Clin Pharmacol Ther 81: 298-304.
18. Fu H, He J, Mei F, Zhang Q, Hara Y, et al. (2009) Lung cancer inhibitory effect of epigallocatechin-3-gallate is dependent on its presence in a complex mixture (Polyphenon E). Cancer Prev Res (Phila) 2: 531-537.
19. Chang PY, Mirsalis J, Riccio ES, Bakke JP, Lee PS, et al. (2003) Genotoxicity and toxicity of the potential cancer-preventive agent polyphenon E. Environ Mol Mutagen 41: 43-54.
20. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRsearch.cfm?CFRPart=312.
21. https://www.fda.gov/media/71180/download.
22. Pamplona FA, Da Silva LR, Coan AC (2018) Potential clinical benefits of CBD-rich cannabis extracts over purified CBD in treatment-resistant epilepsy: Observational data meta-analysis. Front Neurol 9: 759.
23. https://www.gwpharm.com/healthcare-professionals/sativex
24. Chen Z, Harrington PB, Baugh SF (2018) High-throughput chemotyping of Cannabis and hemp extracts using an ultraviolet microplate reader and multivariate classifiers. J Analysis & Test 2: 210-222.
25. Tang Y, Harrington PB (2019) Non integer root transformations for preprocessing nanoelectrospray ionization high-resolution mass spectra for the classification of Cannabis. Anal Chem 91: 1328-1334.
26. Wang YF, Vorsa N, Harrington PD, Chen P (2018) Non-targeted metabolomic study on variation of phenolics in different cranberry cultivars using UPLC-IM-HRMS. J Agr Food Chem 66: 12206-12216.
27. Wang XY, Harrington PD, Baugh SF (2017) Comparative study of NMR spectral profiling for the characterization and authentication of Cannabis. J Aoac Int 100: 1356-1364.
28. Harrington PB, Wang X (2017) Spectral Representation of Proton NMR Spectroscopy for the Pattern Recognition of Complex Materials. J Analysis &Testing 1: 1-13.
29. Wang XY, Harrington PD, Baugh SF (2018) Effect of preprocessing high-resolution mass spectra on the pattern recognition of Cannabis, hemp and liquor. Talanta 180: 229-238.
30. Wold S (1976) Pattern-recognition by means of disjoint principal components models. Pattern Recogn 8: 127-139.
31. Biancolillo A, Marini F (2018) Chemometric methods for spectroscopy-based pharmaceutical analysis. Front Chem 6: 1.
32. Wise BM, Gallagher NB (1996) The process chemometrics approach to process monitoring and fault detection. J Process Contr 6: 329-348.
33. Sampaio BL, Edrada-Ebel R, Da Costa FB (2016) Effect of the environment on the secondary metabolic profile of Tithonia diversifolia: a model for environmental metabolomics of plants. Sci Rep 6: 29265.
34. Motta EVS, Sampaio BL, Costa JC, Teixeira SP, Bastos JK (2019) Quantitative analysis of phenolic metabolites in *Copaifera langsdorffii* leaves from plants of different geographic origins cultivated under the same environmental conditions. Phytochem Anal 30: 364-372.