Available online on 15.06.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Review Article

Phytopharmaceuticals: An emerging platform for innovation and development of new drugs from botanicals

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ABSTRACT

A phytomedicine, or phytopharmacutical, is a complex mixture derived from plant sources that is used as a medicine or drug. The Ministry of Health has begun the standardization of the names of all phytopharmaceutical preparations. The development of phytopharmaceutical products, which might partially substitute some of the conventional medications demanding imported raw materials, and which could be produced by pharmaceutical industries based in developing countries through joint projects. Highest demands are made on clinically proven phyto-pharmaceuticals. Their effect and safety have to be verified in randomized, double-blind, (placebo)-controlled clinical trials. They are developed and scientifically evaluated in the same way as conventional medicinal products. Globally, herbal medicine has been considered an important alternative to modern allopathic medicine. While they have become very popular, only select herbs have been scientifically evaluated for their potential in medical treatment. The new drug Veregen (Polyphenon E) Ointment is the first prescription botanical (herbal) drug approved by the U.S. FDA under the "new" drug amendments of 1962 that required drugs to be proven safe and effective prior to being marketed in the U.S. Because of the unique health benefits and relatively low side effects, natural products such as food/dietary supplements, nutraceuticals and herbal medicines have been gaining popularity all over the world The gap between the popularity of these remedies and the frequently weak scientific basis of their use is striking. In reality, the efficacy and true frequency of side effects for most herbal medicine products is not known because the majority have not yet been tested in large clinical trials and because pharmacovigilance systems are much less extensive than those in place for pharmaceutical products. In contrast to the popularity of herbal medicinal products, physicians and consumers often have a very critical view of robust efficacy and safety profiles. Application of pharmaceutical nanotechnology for plant actives and extracts, is gaining a tremendous growth and interest among the scientist.

Keywords: Phytopharmaceuticals, Harbal, plant extract, phototherapy, Morphine, Ayurveda, metabolites, fingerprint.

Article Info: Received 06 May 2019; Review Completed 03 June 2019; Accepted 10 June 2019; Available online 15 June 2019

Cite this article as:

Bhusnure OG, Shinde MC, Vijayendra Swamy SM, Gholve SB, Giram PS, Birajdar MJ, Phytopharmaceuticals: An emerging platform for innovation and development of new drugs from botanicals, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):1046-1057 **http://dx.doi.org/10.22270/jddt.v9i3-s.2940**

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INTRODUCTION

Herbal medicinal products are pharmaceutical products of plant origin, also known as phyto-pharmaceuticals. They exclusively contain one or more herbal substances or herbal preparations as active ingredients or combinations of them (1). In contrast to synthetic drugs containing chemically active substances, they consist of a plethora of natural compounds that activate or modulate various target systems in an organism. Herbal medicinal products can be variable in their composition. Therefore, to obtain consistent efficacy and safety, standardised medicinal plant extracts are being used. Evidence-based phytotherapy makes use of scientifically verified efficacy and safety by randomized controlled clinical trials. In general, herbal medicinal products are better tolerated and provide a superior benefit-risk ratio to synthetic drugs. Therefore, evidence-based phyto-pharmaceuticals increasingly mentioned in clinical practice guidelines are first-line therapy in various diseases and indications.

The term phyto-pharmacon/phyto-pharmaceutical is derived from the Greek designations *phytón* for plant and *phármakon* for medicine. Basically, these are herbal remedies which are prepared from herbal substances like dried plant parts such as leaves, blossom, herb, bark, or the roots traditionally already known to cure undesired conditions or illnesses. Today, most herbal preparations are

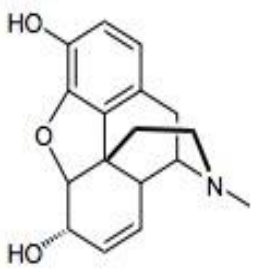
extracts from herbal substances in optimized form with known extraction solvent, drug-extract ratios and processing steps finalized as pharmacologically active dry extract.

Highest quality (GMP), clinically confirmed efficacy and best tolerability and safety of standardized or quantified medicinal plant extracts are key measures for a successful phytotherapy approach. Other than food supplements or botanicals phyto-pharmaceuticals have to follow comparable regulatory guidelines as conventional drugs. Therefore, a high level of scientific evidence supports their efficacy and safety. For example, the St. John's wort extract Ze 117 shows comparable efficacy as the synthetic antidepressant drugs imipramine or fluoxetine (SSRI) in the treatment of depressive disorders (2, 3). St. John's wort (*Hypericum perforatum* L.) extracts have been attested with the well-established use status by the EMA (4) and have reached Level A evidence (5) of efficacy as they confirmed to be superior to placebo in patients with major depression. Further, they are similarly effective as standard antidepressants and have fewer side effects as concluded in a Cochrane meta-analysis (6).

Importantly, evidence-based phyto-pharmaceuticals should be clearly separated from alternative or complementary therapeutic approaches such as homeopathy.

Phyto-pharmaceuticals are complex mixtures

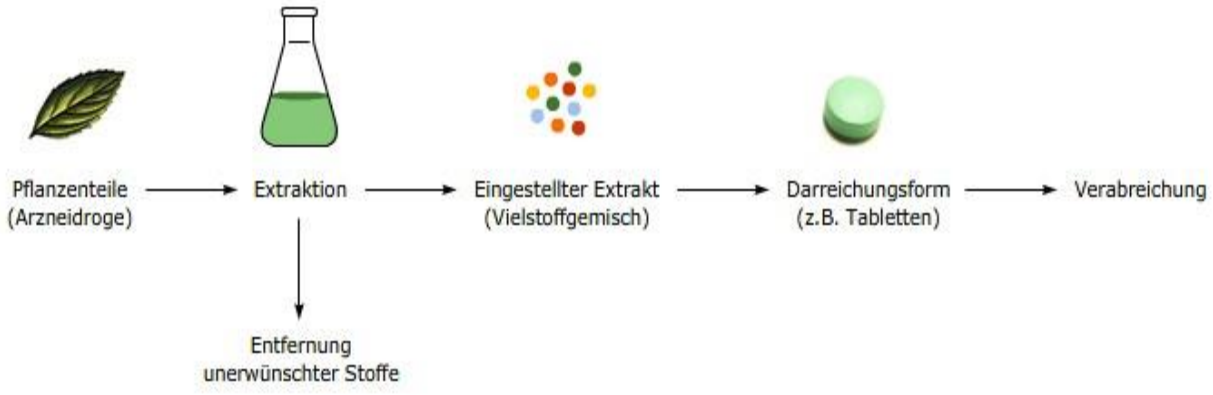
Unlike synthetic drugs, which are single active chemical substances, phyto-pharmaceuticals contain complex mixtures of hundreds of natural components. Among them, several isolated lead substances are well-known to be pharmacologically active while others are still under scientific investigation. The efficacy of herbal medicinal products results from complex interactions the active components with molecular target structures, such as receptors, enzymes, and transport systems. Herbal medicine was also the origin of conventional academic medicine. Even today, about 70% of medicines contain active ingredients that were originally derived from natural substances. Many classical active components, such as the pain reliever morphine, the cardiac glycoside digoxin or the anticholinergic agent atropine originate from plants. Today, however, such isolated substances are no longer considered as phyto-pharmaceuticals as they are either produced by chemical synthesis or are isolated and purified to be used as single substances.



Morphine

The extract as active principle

Phyto-pharmaceuticals are natural products and like other natural products, such as coffee, wine or cocoa, their quality depends on many factors starting from the raw material. For example the plant variety, cultivation, climate, harvest time, drying process and further processing steps can all affect the quality of the final preparation. It is therefore obvious that active ingredients in two different teas made from the same plant type can be present in very different concentrations. Similarly, no coffee tastes the same as another, although they are all prepared from coffee beans. For this reason, increasing numbers of extracts are being manufactured, which are standardised or quantified with regard to important components. Such extracts therefore always contain defined amounts of these active ingredients. Likewise undesired substances; those that cause side-effects, can be removed. Extracts from different manufacturers can thus only be compared with each other to a limited extent. Various different dosage forms, such as film-coated tablets, drops, or ointments, can be manufactured from the extracts.



Phyto-pharmaceuticals are not homeopathic products

Phyto-pharmaceuticals contain pharmacologically active ingredients that interact with protein structures in the human body, the so-called drug targets. They are, therefore, substantially different to homoeopathic remedies, which are highly diluted so that little or nothing of the initial active ingredient is actually left. In contrast to phytotherapy, homeopathy has no solid scientific evidence and is considered an alternative, complementary therapy approach. The concept of homeopathic products is fundamentally contrary to that of modern medicinal products.

Clinically proven phytotherapy

The highest demands are made on clinically proven phyto-pharmaceuticals. Their effect and safety have to be verified in randomized, double-blind, (placebo)-controlled clinical trials. They are developed and scientifically evaluated in the same way as conventional medicinal products. This is very

different to traditional phyto-pharmaceuticals, where the use is primarily based on experience, for example the administration of tannin-containing black teas for diarrhoea.

- Typical examples of clinically proven phyto-pharmaceuticals are:
- John's wort for the treatment of depressive moods (2-4, 6)
- Butterbur for the treatment of hay fever (7)
- Gingko for the treatment of declining mental performance (8)
- Black cohosh for the treatment of menopausal symptoms (9)
- Hawthorn for the treatment of cardiac symptoms (10)
- Valerian and hops for the treatment of sleep disorders (11)

Good Tolerance

In principle, phyto-pharmaceuticals show high tolerability and safety but can bear the same risks as other medicinal products. They can have contraindications, there is a risk of undesired side-effects and drug interactions are possible. However, in general phyto-pharmaceuticals are well tolerated and often have a lower risk potential than synthetic chemical medications. Phyto-pharmaceuticals have a broad therapeutic margin and thus work well for simple and chronic symptoms. Due to their high tolerability and rather low interaction potential, they are well suited for elder patients with multiple medications.

Phytopharmaceuticals – fighting disease with natural substances

Phytopharmaceuticals are herbal medicines whose efficacy is down to one or several plant substances or active ingredients. They have been used for treating diseases since time immemorial. This traditional knowledge is still the basis for many medicinal products made from plants or parts thereof. Herbal medicines have been produced in Baden-Württemberg for many generations.(12)

Plants produce an incredible variety of natural compounds. It is therefore not surprising that humans make use of this huge diversity. Historical sources show that the use of medicinal plants goes way back to the Bronze Age. Europe has a culture of using medicinal plants that starts with Hildegard von Bingen, continues with Friedrich Sertürner who was the first to isolate morphine in pure form and ends with the modern-day production of herbal medicines.

Phytopharmaceuticals are made from secondary plant metabolites

What determines whether plants are suitable as food and for use as remedies for humans and animals? All plants function with a so-called primary metabolism that produces and degrades amino acids, fats, carbohydrates and nucleotides. These synthesis pathways are therefore present in all plants and also quite similar between different plant families. The so-called secondary metabolism is connected to the primary one as it uses building blocks from the primary metabolism to produce a large number of specialised compounds. These are classified into a very small number of groups according to their biosynthetic origins and chemical structures.(13)

Alkaloids' and amines are formed from amino acids. Other secondary metabolites include polyketides, steroids and phenylpropanoids. Around 80,000 unique chemical structures have been isolated from secondary higher plant metabolites.1 Different plant families produce different secondary metabolites, which also vary considerably in their chemical structure, resulting in a huge number of closely related structures. Plants produce different secondary metabolites in different developmental phases. Secondary metabolites are important for communicating and interacting with other organisms and with the environment. Tomatoes are an excellent example for illustrating the effect of certain secondary metabolites as defense barriers. The glycoalkaloids tomatine and dehydrotomatine protect green tomatoes from being eaten by herbivores and from infection by fungi and lichens. They are gradually degraded as the tomatoes mature. Ripe tomatoes contain little or no tomatine and dehydrotomatine and are safe to eat. Some secondary plant metabolites are formed only when the plant is infested by microbial pathogens. These so-called phytoalexins have an antimicrobial effect.(12),(13)

Active plant substances for treating congestive heart failure

The example of tomatoes shows that active plant ingredients are not necessarily individual active ingredients, but a mixture of several. Only a few herbal ingredients are used for therapeutic applications without further processing. Digitoxin is one such compound. It is isolated in pure form from purple foxglove (Digitalis purpurea) and is effective as a cardiac glycoside for treating congestive heart failure. However, in the majority of cases natural compounds are used as models for chemically synthesized pharmaceutical substances: salicylic acid, which is produced by the plant Filipendula ulmaria (commonly known as meadowsweet), and a salicylic acid derivative, acetylsalicylic acid, which is produced by willows, have a long tradition of reducing pain and fever, but have been produced using chemical methods for over a century.(14)

Globally, herbal medicine has been considered an important alternative to modern allopathic medicine. Although the herbal medicines are very popular in the society, only few medicinal herbs have been scientifically evaluated for their potential in medical treatment. In most countries, the herbal drugs are poorly regulated and are often neither registered nor controlled by the health authorities. The safety of herbal medicines remains a major concern. In the United States, the Food and Drug Administration (FDA) has estimated that over 50,000 adverse events are caused by botanical and other dietary supplements.[15] In addition, for most herbal drugs, the efficacy is not proved and the quality is not assured. The World Health Organization's (WHO) Traditional Medicine (TM) Strategy 2014–2023 focuses on promoting the safety, efficacy, and quality of TM by expanding the knowledge base and providing guidance on regulatory and quality assurance standards.[16] In 2012, 119 WHO member states were regulating herbal medicines.[16]

Herbal medicine products include herbs, herbal materials, herbal preparations, and finished herbal products that contain parts of plants, other plant materials, or combinations thereof as active ingredients.[17] Herbs include crude plant material, for example, leaves, flowers, fruit, seed, and stems. Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins, and dry powders of herbs. Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures, and fatty oils of herbal materials. Finished herbal products consist of herbal preparations made from one or more herbs.

The regulatory scenario regarding herbal preparations varies from country to country.[18]

- Same regulatory requirements for all products.
- Same regulatory requirements for all products, with certain types of evidence not required for herbal medicines .
- Exemption from all regulatory requirements for herbal medicines.
- Exemption from all regulatory requirements for herbal medicines concerning registration or marketing authorization.
- Herbal medicines subject to all regulatory requirements.
- Herbal medicines subject to regulatory requirements concerning registration or marketing authorization.
- In Europe, for the marketing approval,[19] the herbal preparations are classified in three categories as follows:
- Traditional medicinal use provisions ("traditional use") accepted on the basis of sufficient safety data and plausible efficacy.
- Well-established medicinal use provisions ("well established use") demonstrated with the provision of scientific literature establishing that the active substances of the medicinal products have been in well-established medicinal use within the European Union for at least 10 years, with recognized efficacy and an acceptable level of safety a product can be classified under.
- Safety and efficacy data from the company's own development ("stand alone") or a combination of own studies and bibliographic data ("mixed application").

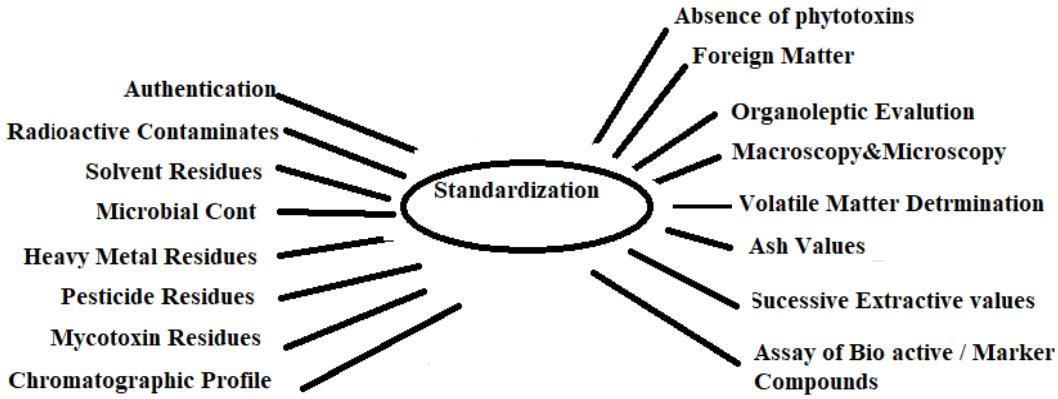


Figure 1: Standardization of phytopharmaceutical

FDA Botanical Drug Development Guidance [20] describes appropriate development plans for botanical drugs to be submitted in new drug applications (NDAs) and specific recommendations on submitting investigational new drug applications (INDs). The term botanical means products that include plant materials, algae, macroscopic fungi, and combinations thereof. FDA guidance recommends that IND must contain sufficient information to demonstrate that the drug is safe for testing in humans and that the clinical protocol is properly designed for its intended objectives.

In addition to general regulatory requirements for an NDA - nonclinical pharmacology/toxicology studies, clinical evidence of efficacy and safety - for botanical drugs there are special requirements to ensure safety and quality of botanicals [20] as follows:

Description of product and documentation of prior human experience

- Description of botanical raw materials used and known active constituents or chemical constituents
- Prior human experience.
- Quality control o Botanical raw materials o Botanical drug substance and drug product
- Identity, chemical characterization, manufacturing processes, biological assay, specifications, stability, current good manufacturing practices, and environmental assessment
- Evidence to ensure therapeutic consistency
- Botanical raw material control
- Quality control by chemical test(s) and manufacturing control
- Biological assay
- Clinical data: Dose-response data and multiple batch clinical data.
- In Indian regulations, the major class of Ayurveda, Siddha, or Unani (ASU) drugs included are:[21]

a. Classical ASU drugs as mentioned in the authoritative books of ASU system drugs, which are manufactured and named in accordance with the formulations described in the authoritative texts. For this category, issue of license to manufacture is based on citation in authoritative books and published literature, unless the drug is meant for a new indication when proof of effectiveness is required.

b. Patent or proprietary medicine makes use of ingredients referred in the formulations of authoritative texts but with intellectual intervention, innovation, or invention to manufacture products different from the classical medicine. For this category issue of a license to manufacture requires proof of effectiveness, based on the pilot study as per relevant protocol for ASU drugs.

In 2010, Department of Ayurveda, Unani, Siddha, and Homeopathy (AYUSH) introduced Rule 158(B) which made the requirement of proof of effectiveness for licensing of a patent or proprietary ASU medicine.[21] This was followed by the release of GCP guidelines[21] for voluntary use by the

researchers interested in taking up clinical trials using ASU medicine.

In India, ASU drugs have been under the purview of Department of AYUSH. In contrast, 2015 regulatory requirements for phytopharmaceuticals are under the purview of the Central Drugs Standards Control Organization (CDSCO).[23] This gazette notification defines regulatory provisions for phytopharmaceuticals and regulatory submission requirements for scientific data on quality, safety, and efficacy to evaluate and permit marketing for an herbal drug on similar lines to synthetic, chemical moieties.

Phytopharmaceutical drug is defined as[22] purified and standardized fraction with defined minimum four bioactive or phytochemical compounds (qualitatively and quantitatively assessed) of an extract of a medicinal plant or its part, for internal or external use of human beings or animals for diagnosis, treatment, mitigation, or prevention of any disease or disorder but does not include administration by parenteral route.[22]

In Schedule Y, the newly added Appendix I B describes data to be submitted along with the application to conduct clinical trial or import or manufacture of a phytopharmaceutical drug in the country.[22] The regulatory requirements for NDA for the phytopharmaceutical drug include standard requirements for a new drug-safety and pharmacological information, human studies, and confirmatory clinical trials. For phytopharmaceutical drug, there is a lot of stress on:

- Available information on the plant, formulation and route of administration, dosages, therapeutic class for which it is indicated and the claims to be made for the phytopharmaceutical, and supportive information from published literature on safety and efficacy and human or clinical pharmacology information
- Data generated on:
- Identification, authentication, and source of the plant used for extraction and fractionation
- Process for extraction and subsequent fractionation and purification
- Formulation details of phytopharmaceutical drug
- Manufacturing process of formulation o Stability data.

The new phytopharmaceuticals regulation permits the development of the drug development using advanced techniques of solvent extraction, fractionation, potentiating steps, modern formulation development, etc.[23]

After NDA approval from CDSCO, the marketing status of the new phytopharmaceutical drug would be like that of a new chemical entity-based drug.[23] The new regulation for phytopharmaceutical is in line with regulations in USA, China, and other countries involving scientific evaluation and data generation.[23]

This new regulation is expected to promote innovations and development of new drugs from botanicals in a scientific way and would help in the acceptance of the use of herbal products by modern medical profession. It would encourage research in phytopharmaceutical drug development for academia, researchers, and industry.

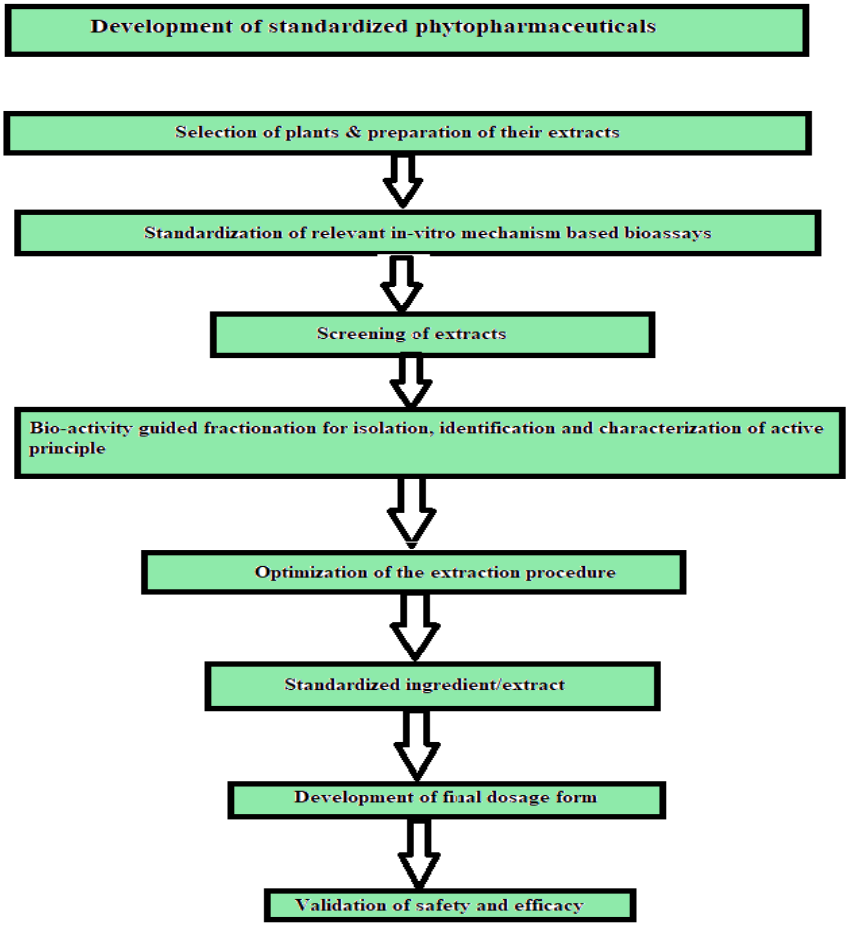


Figure 2: Development of standardized phytopharmaceuticals.

In past year Dr. Tu Youyou won the Nobel Prize in medicine for her discovery of artemisinin isolated from Artemisia annua for malaria. Hope with the new phytopharmaceutical regulation, the Indian scientists would develop noble intentions to discover phytopharmaceutical drugs for unmet medical needs.

Important evidence-based phyto-pharmaceuticals should be clearly separated from alternative or complementary therapeutic approaches such as homeopathy.

Approaches to pre-formulation R and D for phytopharmaceuticals emanating from herb based traditional Ayurvedic processes

Ayurveda, known to have about 5000 years of history, is widely used in India and few other countries. A large part of the formulations used in Ayurveda have herbs/botanicals as ingredients, although some may be made with minerals, metals, and ingredients of animal origin. Ayurvedic formulations are currently industrially prepared into products adopting large-scale technologies that pharmaceutical or food industry uses. Ayurveda mentions a number of processes and dosage forms.[24]

The types of information prescribed in Ayurveda includle an infusion or a decoction of the botanical using water as a medium/solvent, either as a liquid or as concentrated decoction Ayurveda also advocates the use of semi-solid mass (*avaleha*) or dried powder, along with many other processes described in the texts. Such processed material actually forms extracts which in traditional dosages are formulated as pills.

Selection of plant extract as shown below

In certain cases, the infusion or decoction in water is prepared in a dilute solution of a specified herb or herbs instead of water alone, a process referred to as *bhavana*. Materials prepared for any botanical use such a process as prescribed in official texts of Ayurveda as listed in the First Schedule of the Drugs and Cosmetics Act, 1940 of India (or for that matter in TCM or other TM) will come with the known history of safe use (HOSU).

Phytopharmaceuticals are in essence the same as a botanical drug in common parlance and it was the US FDA (United States Food and Drug Administration) that first issued "Guidance to Industry on Botanical Drugs" that paved the way for phytopharmaceuticals to get a status of drug and marketing authorization as a drug after review by FDA US.[25] The definition of the Botanical in this Guidance document differs with the definition of the phytopharmaceutical given above.

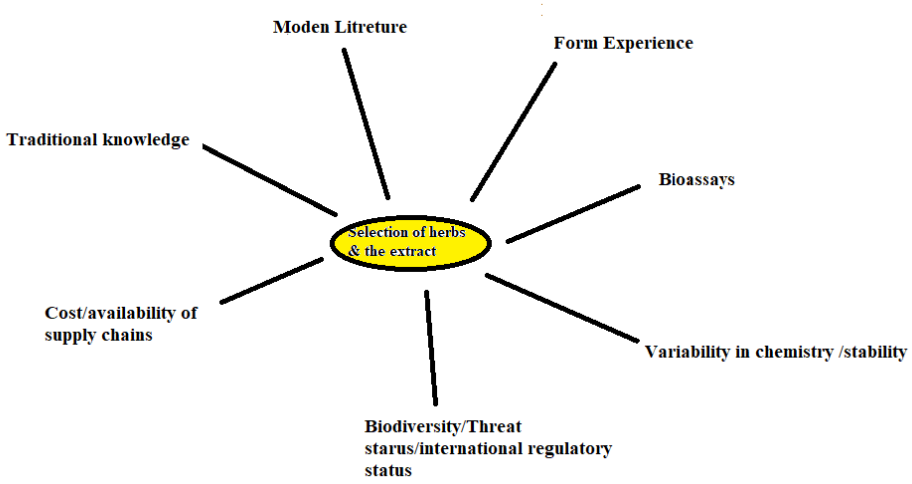


Figure 3: Selection of herbs and the extra

Table 1: Lists a comparison of traditionally processed botanical as per Ayurveda and pharmaceutically processed botanical extracts, adopting decoction process of herbs/botanicals as a case.

Traditionally processed	Pharmaceutically processed
Single boiling & concentration	Multiple boiling-st -Marc-2nd boil-Marc- 3rd Boil combined filtrates
Herb to water =1.4/8/18 Concentration –open pan to 1/4soft extract. Extractive say 35%	Herb to water=1:8 Concentration –closed vessel/ Vacuum/falling film –extractive>35%
Convert soft ext .to dry powder –adsorption on inert material	Convert soft to dry powder –spray drying with or without inert materials/bulking agents
Bhavana extration with /in swaras/ plant/kwatha etc.	Not KNOWN AS PROCESS. but involve washing marc with solvent and adding the washing .
Drying and grinding control on temperature/time/uniformity	High temperature exposure for short time at spray draying/on grinding.

The Drugs Technical Advisory Board of Govt. of India under the Drugs and Cosmetics Regulations (DCAR) has in principle approved notification of provisions in the drugs and cosmetics rules for phytopharmaceuticals as drugs.[26] Drug development using phytopharmaceuticals that bases the leads in traditional knowledge through "Reverse Pharmacology" is gaining attention, and it offers an alternative and perhaps more quicker/economic strategy for drug development.[27]

Traditionally processed as per Ayurveda

One case is selected here for discussion from amongst the many dosage forms made with aqueous extracts in Ayurveda. Ayurvedic process of a herb/botanical as pure extract –*swaras* (fresh juice), *phant*(warm or hot water infusion), *kwatha* or *kashayam* (hot water decoctions) – involves normally a herb to water ratio of 1:4 or 8 or 16 depending on the soft or hard nature of the plant part used. In general, the process describes an open pan treatment and concentration is normally prescribed[24] to one-fourth of the initial amount of water or till one gets a soft extract (*ghana/satwa*). In some cases, such *kwathas* or *ghana*are also adsorbed on an inert material like starch, etc., to get a powder or it may be allowed to dry under sun for a number of days till it forms a powder. This process involves obviously long-time heating and drying, and the controls on this process may vary to some extent batch wise. The dried material is often either soft lumps or in many cases hard lumps which need grinding.

Pharmaceutically processed

Many industrial processors who manufacture an aqueous extract of botanicals adopt large-scale boiling with water, and the process is repeated for each herb three times by filtering the first extract and extracting the mark a second and third time. Most commonly, herb to water ratio is 1:8 during each of the three extraction steps. Combined filtrates are concentrated where a vacuum evaporation technology is adopted including use of falling film evaporators.

Conversion of soft extracts so obtained is generally achieved by spray drying where the extract is exposed to high temperature but for a short time during the spraying step and for not more than an hour during the drying in the cone of a spray dryer. If proper technology is adopted, no step of grinding may be required at the end of this spray-dried material.

Characterizing the material

Adequate scientific studies would be required with an initial step of knowing and documenting in detail all the steps, in both the above processes. While a material prepared as per Ayurvedic process briefly discussed above can be labeled as a HOSU material, it is important to generate scientific data on the pharmaceutically processed material of the same herb produced industrially as per process briefly described above. This is important as the phytopharmaceutical raw-material produced by the pharmaceutical process needs to be similar in characteristics and composition to a HOSU material and such similarity or dissimilarity data would only lead to extrapolatability of the safety profile of the HOSU material to the phytopharmaceutical. This is the first step of R and D before the phytopharmaceutical can be taken up for further formulation development.

For generation of data on the material from the above two processes, a simple conduction of the normal physicochemical parameters, thin-layer chromatographic profiles for comparison, High Performance Liquid Chromatography, HPLC analysis for either qualitative or quantitative data for analytical or biomarkers that normally are adopted by various pharmacopoeia may not be adequate. In these tests, stress is high on understanding the chemistry of processed material involving organic compounds. Many botanicals are known to contain minerals and inorganic compounds in traces to appreciable levels, and for certain pharmacological or therapeutic activities, inorganic compounds also play a great role. R and D studies need to go beyond organic chemistry alone. Process changes in the above two processes need to be carefully documented, and its impact on the nature of the material produced need to be studied. For example, heating at high temperature forms 4-deoxy-11, 12-didehydroandrographolide in *Andrographis paniculata, Nees* [*kalmegh*] that has been reported to be toxic and some pharmacopeia have a test and a limit for the same in the quality specifications. [28] *Bhavana* process of extraction and the impact it does on the nature and quality of extract may be change of pH of the medium/solvent. Can constituents of the *bhavana* herb like "phytosterols if present provide solubalizing effects, or does it add to isotonicity of the medium?", such possibilities have not been studied or reported. If such effects are indeed seen, then the pharmaceutically processed material may also need to adopt the same in industrial scale to be similar to HOSU material.

Limiting the data generation to Thin Layer Chromatography (TLC) profiles or HPLC analytical data may be inadequate in many cases especially when the botanical processed has more compounds that are not chromophoric in nature to provide UV absorption maxima. Principal component analysis (PCA) involving a number of measurement techniques namely Infra red Spectra, Ultra Violet Spectra, Nuclear Magnetic Resonance (IR, UV, NMR) and Mass Spectra (MS) among others offer a great advantage to generate adequately large pool of data that enable to apply statistical analysis. Such an approach offers a high degree of ability to analyze and come to a fair degree of assessment of similarities and dissimilarities between the HOSU material and the phytopharmaceutical. In addition, conducting *in vivo* bioefficacy screening/test protocols involving small animals or organ tissue cultures or cell-line-based assays helps to generate biological efficacy comparison data.

Characterizing through fingerprinting

A good-quality fingerprint cans [29]:

- Provide an objective link between historical data and a proposed new material
- Give an assessment of
- Lab to production scalability
- Raw-material variability (seasonal and regional)
- Extraction robustness
- Sample stability.

Techniques for fingerprint development and its analysis

HPLC is widely available although for a molecule to be detected by UV it must have a chromophores. Evaporative light-scattering detection (ELSD) and mass spectrometry (MS) offer more universal detection methods, but availability and cost can be limiting. Chromatographic resolution is essential to include as many components as possible. With the exception of MS detection, little information on component identity can be obtained. Ultra Performance Liquid Chromatography (UPLC) is an emerging technique with excellent resolving power

Fourier Transfer Infra red Spectra (FT-IR) is quick, relatively cheap, and widely available. A spectrum can give some qualitative information on the general compound classes present along with limited quantitative information. FT-IR spectra can be difficult to reproduce and are low resolution, which can result in small changes being missed.

^{1}H-NMR has the potential to detect any molecule containing hydrogen, thus making it highly universal. High-resolution instruments(>500 MHz) have sufficient sensitivity and resolution to give quality fingerprints for complex natural products. The spectra are qualitatively and quantitatively information rich, but the technique is expensive and not widely available.

Analysis of fingerprints using principal component analysis

Analytical results from multiple techniques can be combined to create a holistic fingerprint prior to input into a PCA model. PCA modeling improves with increased sample numbers. By identifying the techniques and subsequently the data points responsible for the variance, these components can be targeted for identification or monitored as part of a specification.

Pre-formulation R and D

Once having established the similarities of the phytopharmaceutical to the HOSU material, the pre-formulation development work begins. Unlike pre-formulation studies in a pharmaceutical based on synthetic drugs where large guidance and techniques are available, the area for phytopharmaceutical is bereft of the same. Knowledge on such studies seems to be residing in industry due to obvious reasons of confidentiality of such strategy. Creative adoption of many measurement techniques is needed to study the properties of the phytopharmaceuticals, so as to know the ability to obtain quality solid dosage form formulations.

Some examples of the common attributes that need to be studied and suggestions are listed below, although these are non-exhaustive. For other tests/protocols, reference to the various editions of Ayurvedic Pharmacopoeia of India[24] is recommended.

Solubility and re-solubility/re-dispersability: The pharmaceutically processed powder (referred as powder in the paras below for brevity) materials would have undergone some changes physically that render them not easy to dissolve or re-dissolve if needed for making syrup. Generally, the authors have experienced dissolution of about 90-95% of the powder in water even with some warming. Knowledge of the extent of solubility is important to decide the need for additional steps of "adjustment to get the right quantum or, need for a step of filtration during manufacture."

Hygroscopicity: Most herbal extracts exhibit a fair degree of hygroscopicity although the opposite phenomenon "efflorescence" is not uncommon. Hygroscopicity information and extent of the same are very important in formulation of solid dosage forms, as it will have serious implications of the manufacture steps, environmental controls on the manufacturing, design of packs and packaging, and most importantly on the stability and microbial qualities of the material and formulations.

A creative method for such a study would be to expose weighed quantities of the powders whose initial moisture contents are determined, in open Petri dishes to high humidity atmosphere. Samples from these Petri dishes can be drawn at frequent intervals and tested for moisture content and this testing can be continued till one reaches the "critical moisture content (CMC)" for that powder. CMC is that level of moisture when the powder starts to show changes in color/odor/nature (become cohesive, pulpy).

Some extracts may show opposite tendencies-lose water/volatile materials. In such cases, the same testing will provide information on the weight loss rather than weight gains of a hygroscopic powder.

One also needs to know what happens when moisture is absorbed does the material swell? Does the particle size enlarge/ grow? Observing samples of the powder-initial sample and that which has reached CMC under a microscope can provide good information.

Swelling index: Many phytopharmaceuticals exhibit swelling properties and such a behavior will have impact in the steps involving granulation, or even after the powders are filled in capsules or compressed to get tablets. Swelling of the contents of the dosage forms can lead to serious quality problems including breaking of tablets, de-shaping of capsules, change in bulkiness color, etc., of granules. Swelling property is easily testable adopting Pharmacopeial method [30] given in monograph of for *Isabgol* (psyllium husk).

Binding properties: Phytopharmaceutical produced by spray drying process often show varying ability to bind. The powders may vary from amorphous, fine to granular powders; vary from cohesive powders to free flowing powders, with low or high inherent binding capacities. Knowledge of the same is extremely important and measurement of bulk density –untapped/tapped can provide some information. Large difference indicates more air/intra-particle voids. Powders with such large voids may show both, good inherent binding property or sometimes exactly the opposite. An experienced scientist can gauge the binding ability by just pressing some powder between fingers.

Crystalinity and size, amorphous/crystalline: Powders may appear to be crystalline or amorphous to the naked eye. Not much information is available on this property and its impact on both pharmaceutical properties and pharmacological/therapeutic activity. Simple viewing under polarization microscopy or in specific cases, testing by powder X-ray diffraction is suggested. Determination of particle size distribution by normal methods of sieve analysis is suggested.

Interaction with Excipients:

By an amendment to DCAR-Rule169, use of Permitted Excipients has been notified to assist in formulations of Ayurvedic dosage forms.[31] Selection of Excipients follow the normal dictum of compatibility with the powder. They should aid in improving the pharmaceutical properties like binding, lubricating, anti-sticking, anticaking, stabilizing, and solubilizing. The normal dictum that," the Excipients used should not interfere in the method of analysis" that is generally stated in the pharmacopoeia, needs a review in the context of phytopharmaceuticals as the type and nature of analysis done for phytopharmaceuticals is not the same as that which is done for a synthetic drug formulation. This applies greatly to the preservatives used with formulations involving a phytopharmaceutical. Also the recognition that all Excipients may not be necessarily "inert" is required. For example, in case of the phytopharmaceuticals formulated with magnesium oxide/magnesium carbonate as an anticaking agent, which may show slight "laxative effect or potentiate such effect if the phytopharmaceutical also is originally known for laxative effect."(32)

These are but few examples of the area and more need to be studied to obtain the right

formulation with a phytopharmaceutical that mimics/is similar to the traditionally processed formulation, yet it is contemporary, pharmaceutically processed, and developed adopting industrial scale pharmaceutical technology. Greater funding for such research and development by the national and international agencies is the need of the hour apart from creating awareness of such research needs. (10)

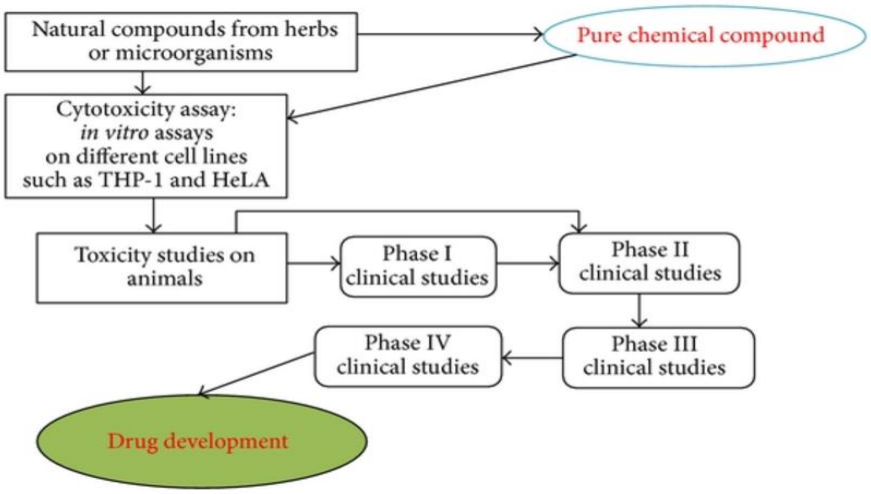


Fig No 4: Herbal product as phytopharmaceutical: steps involved in the process of drug development.

Plant-derived drugs launched (2000- 2010)

Table 2: Plant-derived drugs launched (2000- 2010)

Year	Trade name	Lead Compound	Disease area	Class
2000	Arfadin	Artemisinin	Antimalarial	Sesquiterpene lactone; Semi-synthetic NP
2002	Reminyl	Leptospermone	antityrosinaemia	NP-derived
2002	Apokyn	Galantamine	Alzheimer's disease	Alkaloid; NP
2004	Spiriva	Morphine	Parkinson's disease	Alkaloid; semi-synthetic NP
2005	Sativex	Atropine	Chronic obstructive pulmonary disease	Alkaloid; semi-synthetic NP
2007	Vyvanse	Dronabinol/ Cannabidol	Pain	Cannabinoids; NP
2008	Relistor	Amphetamine	Attention deficit hyperactivity disorder	Amine; NP-derived
2009	Qutenza	Capsaicin	Pain	Vaniloid; NP
2013	Crofelemer	Oligomeric proanthocyanidin	Antidiarrheal Agents	Polyphenolic; NP

Regulatory Challenges in Herbal Product Registration

Regulatory Challenges

- Standardization of Products
- Collection strategy
- Controls on adulterants
- Reproducibility
- Safety & Efficacy

Standardization of Products

Herbs are natural products and chemical composition varies depending upon factors:

- Botanical species.
- Used chemotypes.
- Anatomical part used (seed, flower. Root, leaf etc).
- Storage: Sun, humidity, type of ground time of harvest.
- Concentration of active principle or Biomarker.
- Ascertain consistent chemical profile and biological activity.

Need validation of above as part of regulatory process as lack of information about composition can turn to be black box for the herbal based treatment.

Collection Strategy

It is observed that concentration of bio-marker and active principal varies due to:

- Weather conditions.
- Soil quality.
- Ground water and Moisture content.
- Season etc.

To ensure consistent quality product:

- Need selection and validation of Collection strategy from wild or Good validated harvesting practices.

• Need good manufacturing practices for extraction modes and relate Control on Adulterants Regulatory approval require to ascertain control of adulterants which can be found in the herbal material through different sources:

• Pesticide residue (more likely if it is harvested in the agricultural fields)

• Aflatoxin content

• Bacterial/fungal growth

• Heavy metal contamination The control need to be demonstrated from collection through different processing including extraction and final product manufacturing d parameters.

Reproducibility

One of the biggest challenges due to variation in chemical profile due to various factors which can lead to possible variation in biological activity:

• Must to identify and characterize the bio-marker or active principal (constituent).

• Repetitive testing different batches to control batch to batch variation.

• Establish the relation of active marker or constituent through pre-clinical and clinical studies.

• Standardize collection strategy and validate the process to reduce the variability.

• Develop appropriate analytical method to analyze the bio-marker or active principal.

- Processing of extract or Phytopharmaceutical (if used as an extract)
- Extraction methods, physico-chemical tests, microbial loads, heavy metal contaminants, chromatographic finger print profile with phytochemical reference markers, assay for active constituents or characteristic markers, if active constituents are not known.
- Data on the active Phytopharmaceutical establishing the natural window of constituents (marker compounds) reflecting geographical and seasonal changes in a year, wherever applicable.
- Information on any excipients (diluents or builders or stabilizers or preservatives used, if any) and their proportions.
- Details of packaging of the extract or Phytopharmaceutical, storage conditions and labeling.

  • Formulation of Phytopharmaceutical drug applied for:

- Composition, dosage, excipients, stabilizers, agents used, packaging materials, test(s) for the identification for the Phytopharmaceutical.
- Quality specifications for actives and inactive Phytopharmaceutical, chromatographic finger print profile with phytochemical reference maker(s) and assay for active constituent(s) or characteristic maker(s), if active constituents are not known.

• Manufacturing process of formulation

• Stability data : for 0, 1, 2, 3 and 6 months

• Safety and pharmacological information

Provide comparative chemical and chromatographic profile, and spectroscopic information

Safety and Efficacy

• There is very limited scientific evidence to establish the safety and efficacy of the herbal products.

• Out of top herbal products which have scientific evidence of efficacy has some concern over safety.

• More advance technologies need to be used to characterize the herbal products to control the variance and establish the safety and efficacy.

• Regulatory need more scientific / evidence based medicine which are designed through pre-clinical and clinical trial studies instead of tradition or supposed millenarian belief.

• Need more funding for research and understanding of herbal products to meet regulatory requirements.

• Current regulation of standard medical therapies can not be applied to herbal products, may need regulation which is suitable to herbal products.

• **Animal toxicity and safety data** :

- 28 to 90 days repeat dose oral toxicity on two species of animals of which one should be non rodent as per Schedule Y;
- In-vitro Genotoxicity data (Ame's test and Chromosomal aberration test as per Schedule Y);
- Dermal toxicity tests for topical use products;
- Teratogenicity study (only if Phytopharmaceutical (s) is intended for usage during pregnancy).

Pharmacological Activities of Phytomedicines: A Challenge Horizon for Rational Knowledge

On a global perspective, we are yet in need of a rational, complete, and widely accepted theory to explain phytotherapy efficacy and the mechanisms of action inherbaldrugs. Forthispurpose,anewapproachbasedonthe"-omics"couldproveextremelyuseful.

The "Modern Medicine Approach" Was Not Enough

A great number of herbs have been analyzed under the approach described previously, and a compound or group of compounds have been proposed as the biologically active agents that play the key role in the pharmacological effect [32]. Sometimes, this approach has resulted in the ability to discover compounds with great selectivity for a chosen target [33],which can be as diverse as enzymes, receptors, antibodies, or signal cascades, but generally, herbal extracts are a mixture of bioactive or inactive compounds, and it has been suggested that the biological activity of herbal medicine results from the combination of different active components [34]. Systems biology has revealed that human cells and tissues are composed of complex, networked systems with redundant, convergent, and divergent signaling pathways, with different possibilities of inter action when such agreat number of compounds converge [33]. The combination of effects is described with the terms add it,synergy, and antagonism, although both synergy and antagonism can be defined in relation to an additive expectation. Strictly speaking, synergism, which was advanced in a landmark paper by Williamson [35] and further outlined by Ulrich-Merzenich et al. [36], is defined by a combined effect of substances that is greater than would be expected from the individual contributions [37]. Sarris et al. [38] defined it as a "super-additive" biological effect when

combined, as opposed to being just the sum of their individual parts or additivity (e.g., 1 + 1 = 5, not 2). Antagonism, or "negative synergy", on the contrary, appear sifit works backward sandared effect is achieved.. According to investigations carried out by Einbond et al. [39], the inhibitory effect on Na+-K+-ATPase activity, compared with the summation effect obtained by each one administered alone, is higher when co-administered. In this case, this observation is an example of pharmacodynamic synergy because actein or digitoxin facilitates the action of the other in the same target. Synergy can also be related to pharmacokinetic parameters. For example, permeability values and the absorption rate of some drugs can be altered. Coumarins can increase the active absorption via carrier-mediated proteins (i.e., of flavonoids) and inhibit P-gp-mediated efflux systems .That is exactly what happens in a plant extract. The ingredients with little or no direct activity on the pathogenic process may assist the "actives" to reach the target, either by improving bioavailability or decreasing the metabolism and excretion of the active principle [41], and in the overall pharmacological effects and the therapeutic efficacy of multi-extract preparations, constituents are involved synergistically [42]. In the same sense, the complex matrix in a plant can also exhort protection of the active principles, through antioxidative and other protection actions [43]. The expected overall effect is not easy topredict based on the known effects of the different substances in an individual herbal preparation assuming that each component acts on different pharmacological targets. In the case previously described (actein/digitoxin), not only does Na+-K+-ATPase activity increase, but the expression of NF-κB promoters, p-ERK, p-Akt, and cyclin D1 protein levels are also affected.

The treatment of diseases with mono-substance therapy, "the silver bullet concept", in other words, with a characterized compound and a perfectly defined mechanism of action at a particular dose that fulfills the positive (efficacy/stability) and negative requirements (such as toxicity) has been inherited from classical pharmacology and assumed by phytotherapy in an attempt to achieve rationality. However, especially in the treatment of chronic diseases, it is increasingly viewed as inadequate in many clinical situations due to ineffectiveness, resistance problems, and the side effects that may occur [44]. For these reasons, the herbal medicinal preparation derived from several herbal plants that contains a large number of secondary phytochemical compounds has been seen as feasible for chronic therapies. The rationale behind combinations is frequently questioned, and it remains challenging to assess the individual contribution of each of the combination partners to the over all activity of the preparation[44,45].

The "-Omic" Technologies; Present and Future Epigenetic studies are already demonstrating that combinations of constituents do not only have the added effect of triggering an increase in the number of expressed genes; they do in fact trigger new genes altogether [38]. The mechanism underlying phytotherapy efficacy is, clearly, much more sophisticated. A good example of this can be found in the work of Ulrich-Merzenich et al. [36], who showed a summary map of genes expressed in human chondrocytes stimulated with willow bark extract STW-33-1 (30 µg/mL and 50 µg/mL) as well as with quercetin (10 µM), diclofenac (30 µg/mL), and acetylsalicyclic acid (50 µg/mL). Undoubtedly, the results demonstrated that each of the substances and extracts, in spite of the fact that all exert anti-inflammatory activity, has a specific gene expression profile, as well as that they will ow bark extract showed a different gene expression profile depending on the dose administered. To study this further, a similar map of genes expressed in the blood cells of rats after the animals were treated with willow bark extract STW-33-1 and its different fractions (ethanol, ethyl acetate, and water) was constructed. In this case, as might be expected, the treatment with different fractions of the extract leads to distinct gene expression profiles in vivo as a direct consequence of the different combinations of compounds and their concentration in each fraction. To sum up, it must be concluded that not only is the pattern of gene expression (signature in some texts) different depending on whether a substance or a mixture of substances (extract) is administered, presumably more complex in the last case, but also depending on the substance/extract concentration or the extract fraction administered

The development and refinement of "-omic" technologies, including genomics/transcriptomics, proteomics, and metabolomics, has led to advances in the assessment of both the efficacy and, no less important, safety of herbal medicines [49,50] referred to by some authors as "herbomics", although this term is still in its infancy. An interesting study is the one published by Wang et al. [50], in which the possible toxicological role of Matricaria chamomilla was evaluated using metabolomics approaches. After tea administration in human volunteers (200 mL/day for 2 weeks, made from fresh flowers), urine was analyzed during the dosing period and two weeks later. Its administration results in an increase in the excretion of some metabolites (including glycine and hippurate), while creatinine excretion decreased, with baseline parameters still altered 14 days later. Apart from the therapeutic effect,it is clear that Matricaria chamomillatea modified patients'homeostasis. On that basis, the authors suggest that gut microflora might have been disrupted. Security assessment must include the kinds of techniques able to fulfill differences between the toxicology of a specific compound and that of whole herbs or an extract. Despite the high level of complexity and cost of "-omic" techniques, they are the only way the pharmacological action of many species can be fully understood. They are the key to a rational and fully accepted form of phytotherapy

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