

CHAPTER 19

PLANTS AS SOURCE OF MEDICINES

New perspectives

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Abstract. An enormous variety of medicinal plants are used worldwide by about 80% of the world population, although in most cases no scientific studies have been done to prove the efficacy of these medicinal plants. Considering that most present-day western medicines are based on the traditional medicinal plants of European, Mediterranean and Arabic origin, the variety of plants in use around the world may very well represent an enormous treasure for drug development. To develop evidence-based medicinal plants and novel active compounds from this very important heritage, the present-day reductionist approach of drug development of 'a single compound for a single target' will probably not develop this resource to its full potential. Instead, holistic approaches, such as clinical trials and systems biology, need to be employed, as in many cases activity might be due to pro-drugs which are activated in the body after administration of the medicine or to synergy among compounds. Metabolomics, proteomics and transcriptomics will be important tools for such holistic approaches, requiring proper statistical methods to deal with the large data sets.

Keywords: drug development; natural products; medicinal plants; traditional medicine; systems biology; metabolomics

INTRODUCTION

Medicines, the core of health care, cure diseases (such as antibiotics), relieve symptoms (such as analgesics), are preventive (such as anti-hypertension drugs) or substitute for endogenous compounds (such as insulin). The search for medicines, which undoubtedly began in prehistorical times, has led to compounds such as morphine, atropine, tubocurarine, quinine and digoxin. Indeed, many of the present-day medicines in the western world have been developed on the basis of traditional medicines with receptors and mechanisms of action identified only recently. This identification of receptors has opened ways of screening for novel, bioactive compounds and to design and subsequently synthesize similar structures. Yet, in the history of drug development, society has moved from broad screening and testing on humans towards a molecular-level screening in which tests can be done on a nano-

scale, changing from a holistic empirical approach to a reductionist computational approach (Figure 1).

Test systems in drug development

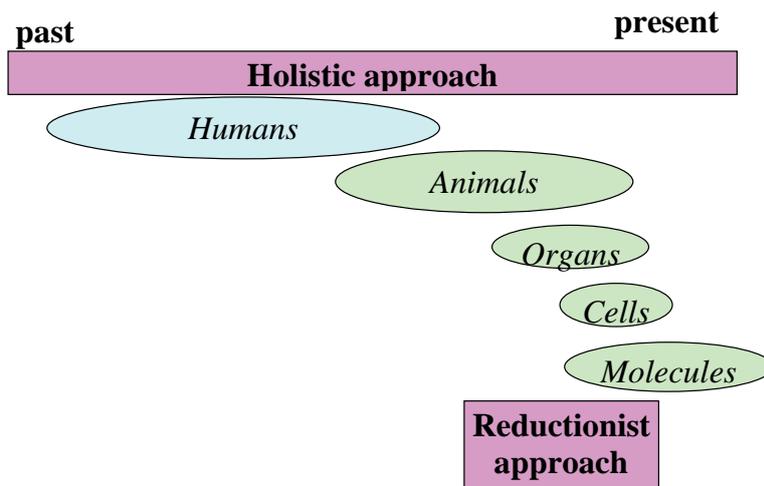


Figure 1. History of drug development since ancient times

Originally, ancestral people most likely tested medicines on themselves and later on patients. With the development of pharmacology as a specialization of medical science a shift to animal experiments occurred. Test systems for medicines were, for example, either the general Hippocratic screening (Malone and Robichaud 1962; Sandberg et al. 1971; Verpoorte and Bohlin 1976) or more specific tests for certain activities, such as for analgesic and anti-inflammatory properties. These tests were further simplified by the use of isolated organs, allowing the testing of even more compounds in shorter time. Due to the improved understanding of mammalian pharmacology and the identification of receptors subsequently drug development focused on molecular targets to further speed-up the screening of compounds for pharmacological activity. Eventually, this resulted in the present-day high-throughput screening methods, which, through miniaturization and robotization, can screen 100,000 samples a day for some targets (e.g., receptor-binding or enzyme-inhibition assays). Moreover, the increasing knowledge about receptors has led to structure–activity studies, enabling medicinal chemists to design novel structures for the same target. For certain drug activities, cellular-based assays are now commonly used, particularly for developing antibiotics and anti-tumour drugs.

A good example of this long route for drug development is probably that of curare (Bisset 1989; 1992; Bovet et al. 1959). When the Native Americans moved into the rainforest of the Amazon some 20,000 years ago, some 50,000 different plant species were around them. From this large population of plant species, they

were able to select two plants that contain dimeric alkaloids that were very toxic when injected but harmless when eaten, the ideal arrow poison for hunting. The curare was studied extensively some 70 years ago with many years devoted to unravelling the structure of the active constituents, complex dimeric alkaloids (Bovet et al. 1959). The mode of action was observed to be a blocking of the acetylcholine receptors in the muscles, causing complete paralysis and death due to failure to breathe. The distance (14 Å) between the two quaternary nitrogen atoms was demonstrated to be essential for the activity (Bovet et al. 1959), a discovery that opened the way for synthetic analogues. This instance of a structure–activity study can be regarded as the first example of the role medicinal chemistry would play in drug development.

PRESENT-DAY DRUG DEVELOPMENT

Despite enormous progress in medicinal chemistry, the development of a novel drug has become more and more difficult. The reasons are manifold, but include the fact that for major diseases good drugs are available, and developing a better drug that is active on the same target and that is not more expensive, becomes increasingly difficult. As a result, in 2003 only 21 novel drugs (novel drug applications, NDA) were brought to the market. Interestingly, of the 877 novel medicines that were developed in the period 1981-2002, 6 % were natural products, 27 % were derivatives of natural products, and 16 % were synthetics developed on the model of a natural product (Newman et al. 2003), demonstrating that nature is an important source for developing novel leads for medicines.

The downward trend in the number of novel drugs is unlikely to change in the near future and the fact that recently some important novel drugs were taken off the market due to serious side-effects means that in the future introducing novel medicines to the market will be even more difficult. Costs for drug development are already thought to be 800 - 1000 million Euros with 10,000 to 100,000 compounds tested to find one novel medicine. The average development time from idea to market is now almost 15 years (DiMasi et al. 2003; Butcher 2005; Dickson and Gagnon 2004).

At present some 450 targets are used in drug development (Drews 1996). Industry believes the chances of finding novel drugs will much improve now that the human genome has been sequenced, offering improved chances for identifying novel targets for drug development. However, quite some basic research will be required in the coming years to understand the role of all potential novel targets for health and disease.

NOVEL APPROACHES

Another possibility of developing drugs, however, does exist. An examination of the history of drug development indicates that most western medicines are based on traditional European, Mediterranean and Arabic traditional medicines with some examples, such as curare and quinine, based on traditional knowledge from other regions. At least 80 % of the world population is estimated to be still using such

traditional medicines in primary health care, including 40,000 to 70,000 medicinal plants (see chapter by Schippmann et al. of this volume) representing about 20 % of all higher-plant species. In most cases, very little is known of these plants used in traditional medicines. A search in NAPRALERT, the most important scientific database on plants and their bioactive constituents, some 10 years ago suggested that only about 15 % of all plant species had been studied to some extent for their phytochemistry and only about 5 % for one or more biological activities (Verpoorte 2000). Although extensive research on medicinal plants is published every year, only a few plants have been comprehensively studied for pharmacological activity. Considering these facts, traditional medicines and medicinal plants obviously represent a great source of novel leads for drug development.

To tap this source for novel drugs, two distinct approaches are available: the reductionist approach (based on the paradigm single target, single compound) used in present-day drug development, and a holistic approach (based on measuring the effect of a traditional medicine in an in-vivo system). To better understand the implications of both approaches, the present-day approach in drug development should be examined first.

The reductionist approach

In a stepwise approach, drug development basically consists of two phases: the first phase concerns the identification of a novel, active compound (usually referred to as 'novel chemical entity'), which will be approved as an Investigational New Drug (IND) for clinical trials (Figure 2). The second phase concerns clinical trials, which exist of three phases (Figure 3) of which, if the drug passes all three, the last step will be the official registration.

The whole process starts with finding a 'hit', defined as "a molecule with confirmed activity from the primary assay (HTS), having a good profile in secondary assays and a confirmed structure". Further studies on the hit and the pharmacological and toxicological effects of the compound and others with related structures, including structure-activity relationships, may result in a 'lead', defined as "a series of hits for which a structure-activity relationship (SAR) is shown and activity demonstrated both in vitro and in vivo". A lead can also be a derivative or (synthetic) analogue of the hit.

To find a hit, a large number of compounds are tested in a primary screen, such as in a cell culture assay (e.g., for anti-tumour compounds), on micro-organisms (for antibiotics), or in a molecular target assay (for enzymes or receptors). The tested compounds can come from large libraries of previously synthesized or isolated compounds, mixtures of compounds obtained by combinatorial chemistry, or extracts from plants, micro-organisms or other organisms. In the case of mixtures, the active compound needs to be identified, usually by bioassay-guided fractionation in case of extracts of natural origin.

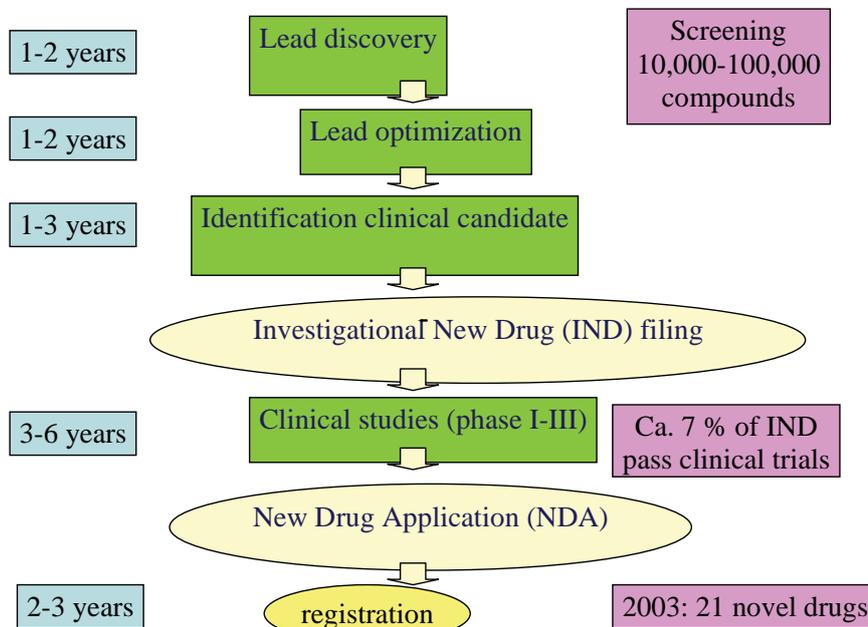


Figure 2. Scheme of present-day drug development

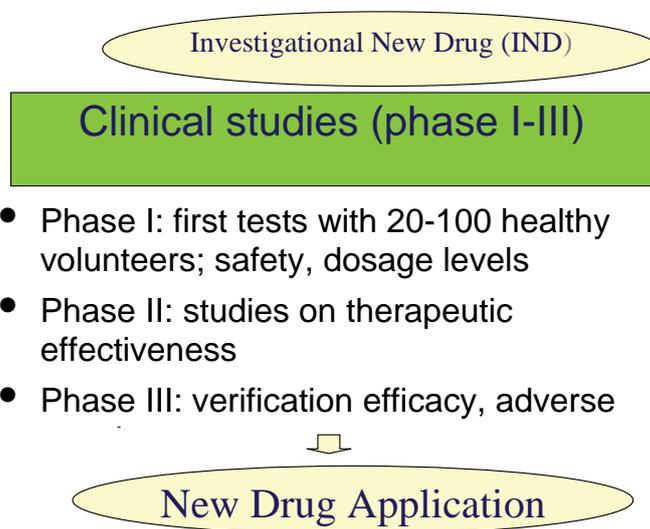


Figure 3. Clinical phases of drug development, which might be applied for traditional medicines

When animal tests or isolated organs are used, the bioassay-guided fractionation is hampered by the fact that the assays require relatively large samples and are time-consuming. With the HTS systems and cell lines much smaller amounts are needed, explaining the reason why tests of natural products for anti-tumour compounds and antibiotics have been quite successful throughout the years. After molecular targets became available, interest in natural-product extracts increased considerably.

Clearly, the analysis by Newman et al. (2003) demonstrated that natural products are a good source for drug development. As described above, about half of all novel drugs developed in the past 20 years are based on natural products. Developing a novel drug from traditional medicines according to the reductionist approach would require the establishment of the affliction for which the traditional medicine is used so that suitable targets could be selected for screening for active compounds. In this, ethnopharmacology necessarily plays a major role in establishing the use of plants as medicines.

The holistic approach

A holistic approach is common in traditional medicine (Chan 2005). The traditional healer makes a diagnosis based on a thorough examination of the patient and then prescribes a personalized medicine, usually consisting of a mixture of different ingredients. This methodology is in clear contrast with most cases of western medicine, where the same dosage is given to all patients. Only recently a more personalized medication is considered in western medicine, due to the development of novel disciplines such as pharmacogenomics and pharmacogenetics, pharmaceutical disciplines concerned with genetic differences and the effect of medicines.

Because of the holistic approach of traditional medicine, a holistic approach to study drug activity of these medicines seems more appropriate than a reductionist approach. Several reasons for a holistic evaluation can be discerned, including the possibility of synergy among plant extract constituents and the occurrence of pro-drugs (the formation of the active compound(s) after intake of the medicine). Both of these factors are realistic assumptions. Synergy has been proven for berberine and the phenolic compound 5'-methoxyhydrocarpine, with the antimicrobial effect of berberine strongly enhanced by 5'-methoxyhydrocarpin, a compound that increases the accumulation of berberine in the cell by inhibiting the multidrug resistance pumps and thus reducing the efflux of berberine from the cells (Stermitz et al. 2000). In addition, with cannabis the combination of cannabinoids has been demonstrated to be more beneficial as a medicine than the major active compound, Δ^9 -tetrahydrocannabinol (THC) (Whalley et al. 2004; Wilkinson et al. 2003; Williamson 2001). THC is also an example of a pro-drug, as THC is only present in the plant as a minor degradation product of THC-acid, a constituent with little bioactivity. By heating the THC-acid (through smoking or brewing a tea), however, the active compound THC is formed. Another interesting example is willow (*Salix*) bark, well-known in Europe as an analgesic and the basis for the development for acetylsalicylate (aspirin), developed more than 100 years ago to treat, among others,

headache (Jack 1997). Aspirin is probably one of the most successful drugs ever made with new applications of this drug continually being discovered, but for which many questions about the mode of action still exist as no single clear target through which activity is exerted is known. Neither acetylsalicylate nor salicylate is present in willow bark; instead, the bark contains salicin, a glucoside from salicylic alcohol (Bruneton 1993). In the body the sugar is split off and the alcohol is oxidized to salicylate, making this a clear example of a pro-drug. With the present-day paradigm of drug development salicylate may never have been found. With glycosides being very common in plants, many examples of pro-drugs may occur in traditional medicines.

Studying traditional medicines in a holistic approach can be done by clinical trials, systems biology, or a combination of the two. In well documented traditional medical systems such as in Asia, the safety of the medicines can be assumed based on the well documented thousands of years of use. Clinical studies can thus easily be done. The first years in drug development spent to identify a target and find a safe lead compound (Investigational New Drug, Scheme 2) as well as the phase-1 clinical trials can be skipped, allowing traditional medicines to enter directly into phase-2 clinical trials. The use of a systems biology approach for drug development has been estimated to reduce the development time of a drug by half and the costs by about 70 % (Butcher 2005). To use such an approach in combination with traditional medicines may even further reduce costs. When activity of a traditional medicine is confirmed in a clinical trial, this could serve as the basis for the registration as a medicine.

A systems biology approach could be important in finding the mode of action and maybe even in finding novel mechanisms of action and/or novel targets. Systems biology aims at understanding biological complexity by unbiased measurements of as many as possible parameters without having any hypothesis. These measurements could be at the level of the genome, transcriptome, proteome or metabolome, as well as on physiological parameters such as blood pressure, pulse, pain and fever. Using suitable bio-statistical methods all these data can be analysed and, for example, correlations between certain parameters can be made. These correlations can be used to identify the occurrence of compounds in extracts and a biological activity (Butcher et al. 2004; Hood and Perlmutter 2004; Lindon et al. 2004; Nicholson et al. 2004; Wang et al. 2004). Such approaches have already been applied for studying toxicity of drugs by the analysis of body fluids such as urine by means of NMR (Holmes et al. 2000; Beckonert et al. 2003; Lindon et al. 2004).

Results from studies of medicinal plants have already been reported. For example, Roos et al. (2004) analysed different accessions and extracts of St. John's wort by means of NMR and correlated the results with activity on the opioid receptor. Wang et al. (2005) showed that chamomile ingestion over two weeks resulted in a clearly different NMR profile of urine metabolites. An interesting example is the work of Boelsma et al. (2004), in which the effect of *Ginkgo* extract on peripheral blood flow in healthy volunteers was studied, demonstrating that some subjects had an increased blood flow, some had decreased blood flow and others no change in blood flow. While one may conclude that this plant apparently has no reproducible activity and thus is not suited as a medicine, a more detailed analysis

showed that those subjects that had no change in the flow had an average normal value before the experiment, whereas the those that had a decreased flow had above-average flows and those with increased flow had below-average flows. Thus, the plant extract normalized the peripheral blood flow. ¹HNMR-analysis of the urine of the treated persons allowed subjects in each of the three groups to be separated from the others by means of multivariate analysis.

Such studies are the first step to a better understanding of the activity of medicinal plants. With some 40,000 to 70,000 plants being used in traditional medicine, a complete analysis of activity will be an enormous task. A major problem is the difficulty in patenting medicinal plants as discovery of traditionally claimed activity would be no innovation. To invest in such studies is thus not inviting, and some sort of legal protection will have to be created for the companies, governments and others that develop traditional medicines into registered evidence-based medicines. Similar to patent or breeder's rights, such legislation would be a major boost for the development of herbal medicine and with that for drug development in general.

METABOLOMICS

A key technology for systems biology is metabolomics. Metabolomics aims at quantitatively and qualitatively analysing all compounds in an organism (Fiehn et al. 2000; Fiehn 2002), a very ambitious goal. While no idea on the number of compounds in an organism is available, in our opinion a good estimation would be that this will be similar to the number of genes. For a plant this would mean that the metabolome consists of some 30,000 compounds, some polar, some non-polar; some in large amounts, some only a few molecules in a plant; some stable and some unstable. Probably the plant with the largest number of identified compounds is tobacco, particularly in the leaves, in which about 3000 compounds have been identified (Nugroho and Verpoorte 2002).

For the analysis of such complex mixtures three methods are available: chromatography (HPLC, GC, electrophoresis, TLC), mass spectrometry (MS) and nuclear magnetic-resonance spectrometry (NMR). The chromatographic methods have the advantage of sensitivity and specificity in case of specific detectors, such as MS. The window for compounds that can be analysed in one single analysis, however, is limited. In addition, a major disadvantage is reproducibility. The ever ongoing quest for even better columns means that one will not be able to secure the same column a few years after having developed a protocol. Mass spectrometry has the advantage of high sensitivity and high selectivity, but the sensitivity may be significantly different for different compounds and can be influenced by the matrix in which one measures, producing problems in reproducibility. In terms of resolution, mass spectrometry is probably the method in which the largest number of compounds can be analysed in one single analysis. For both chromatographic methods and for MS, however, each individual compound has a different detector response and, consequently, for quantitative analysis calibration curves are required for every single compound.

¹HNMR spectrometry is the least sensitive of all the methods, but in contrast to the other methods is highly reproducible, as this method of analysis is a physical measurement of compounds. With the same field strength of the magnet the same spectra will always be obtained. ¹HNMR is the only method that allows direct comparison of the quantities of the compounds present, as the signal intensity is determined by the molar concentration and thus will be the same for all compounds. This means calibration curves are not needed and with a single internal standard, quantitative analysis can be done for all compounds with distinguishable signals in a mixture (Choi et al. 2003; Choi et al. 2004b; Choi et al. 2004c; Hazekamp et al. 2004; Kim et al. 2003; Kim et al. 2004; Schripsema et al. 1991; Schripsema and Verpoorte 1991; 1995; Frederich et al. 2003; Sumner et al. 2003; Ward et al. 2003). The number of compounds that can be measured in a single analysis is smaller than with the other methods, but every compound that has a proton will be detected. By using 2D-NMR the identification of compounds can be facilitated and minor compounds can be better observed. Thus, Bilia et al. (2001) identified a number of compounds in St John's wort extract and Choi et al. (2004a; 2004c; 2004d; 2005; 2006) was able to analyse a number of compounds in tobacco and *Catharanthus roseus* extracts using 2D-NMR methods. The 2D-J-resolved spectra have particularly been shown to be a very useful tool (Choi et al. 2004a; Choi et al. 2004c; Choi et al. 2004d; Choi et al. 2006).

To be able to draw any conclusions about the effect of external conditions on a system, one needs to define and establish normal biological variation (e.g. Choi et al. 2004a; Choi et al. 2004c; Choi et al. 2004d; Choi et al. 2005; Choi et al. 2006). Also, each method requires dealing with large amounts of data. Many plants need to be analysed under different conditions and at different points in time (day/night, young/old leaves and developmental stage, for example). Signals or peaks need to be identified and quantified. For the analysis of such large data sets, chemometric methods, such as multivariate analysis and principle-component analysis, have been shown to be excellent tools, allowing, for example, the comparison of complex spectra or chromatograms and the identification of patterns of co-occurring

Box 1. *Some applications of metabolomics in connection with medicinal plants and natural products*

- Functional-genomics analyses
- Biosynthetic-pathway mapping
- Systems biology studies of traditional medicine
- Safety assessment of GMOs
- Phenotyping for breeder's rights
- Dereplication in identifying active compounds in plants
- Quality control for botanicals, food, wine and other products
- Culture broth analysis in fermentation processes

compounds or clear differences between samples. These conclusions can be matched with data from proteomics and transcriptomics and eventually all these ‘-omics’ may be combined into one data set that can be analysed with statistical tools. The applications of metabolomics are many (Box 1) and will be one of the major tools in further development for quality control, plant breeding and studying bioactivity of medicinal plants.

Metabolic profiling of *Ephedra* using $^1\text{H-NMR}$ and multivariate analysis

Overview of all metabolites, able to differentiate all species

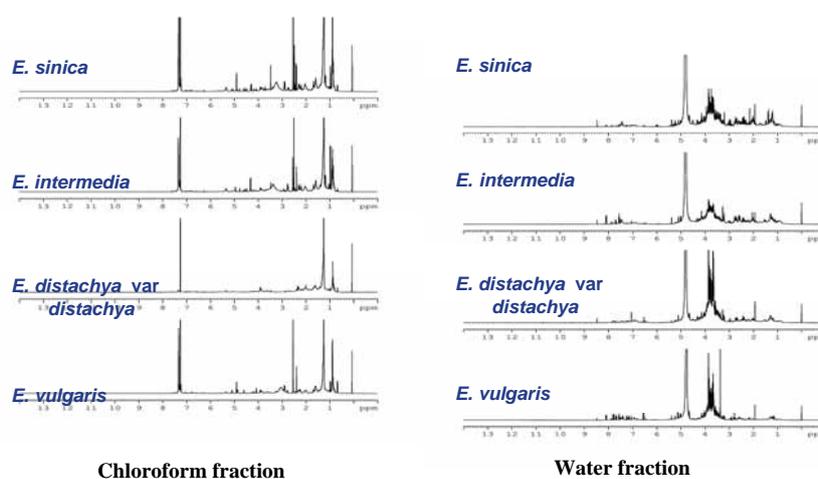


Figure 4. $^1\text{H-NMR}$ spectra of the extracts obtained from different *Ephedra* species collected in Taiwan using the chloroform–methanol–water two-phase extraction method

An example is the application of a metabolomics approach in the quality control of *Ephedra* (Kim et al. 2003; Kim et al. 2005) (Figures 4 and 5). In Taiwan, different species of *Ephedra* occur; *E. intermedia* is the species most commonly used. In western medicine mainly one species is (was) used as medicine, *E. sinica*. By measuring the $^1\text{H-NMR}$ (Figure 4) of a large number of different materials collected from the various species and by principle-component analysis (PCA), one can see (Figure 5) that the *Ephedra* species can be separated on the basis of two principle components, representing a series of signals in the $^1\text{H-NMR}$ that can be used as markers for each of the species. A series of samples bought on the market fell outside the zones of all tested species and did not fulfil the demands for *E. intermedia*. Further analysis indicated that these samples were mixtures of *E. intermedia* and *E. sinica*, nicely demonstrating the strength of this approach. First, by adding more and more data, the typical characteristics of each of the species become more and more evident due to the high reproducibility of $^1\text{H-NMR}$, and all data accumulated during years can be used for the multivariate analysis. Second,

since one looks at all the signals, adulterations by adding compounds, such as ephedrine or other (even related) plants, for example, will be observed.

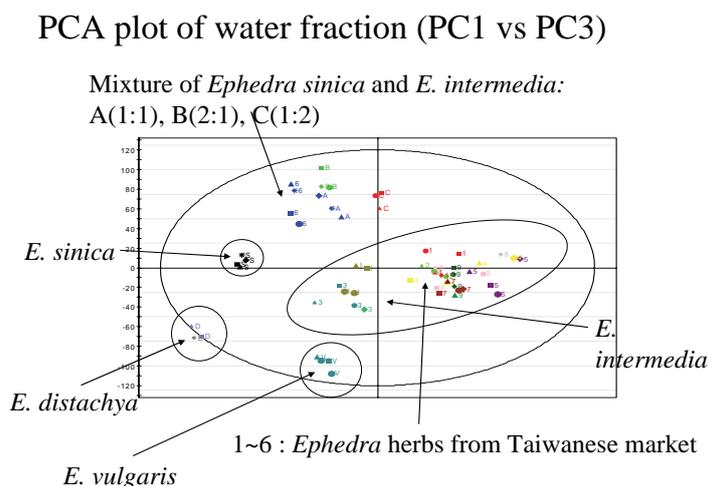


Figure 5. Principle-component analysis of the ^1H NMR spectral data of the various *Ephedra* species (letters) and samples purchased on local markets (numbers)

PERSPECTIVES

Globalization has resulted in a renewed interest in traditional medicine, and expectations are that in the coming years Chinese, Ayurvedic and other traditional medicines will be marketed all over the world using the Internet as the source of information and the place to buy. Regulatory authorities, however, will require evidence of efficacy. To develop evidence-based traditional medicines, I think we need a return to the principles of historical methods of drug development: a holistic approach with patient and animal experiments in the centre and using the latest technology for measuring as many different parameters as possible to prove activity, detect possible toxicity and discover possible leads to the mode of action. This methodology would have great impact on future drug development: back to in-vivo experiments and examination of mixtures, pro-drugs and synergism, which could quite possibly result in finding new modes of actions, new targets and new lead compounds. Such a holistic approach should boost the number of medicinal plants in agriculture and horticulture, as well as their total production volume. Good agricultural practice will be an important item in this connection.

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