

Utility of DNA Profiling In Quality Control Of Medicinal Herbs

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Botanical products are gaining popularity worldwide. The market for Chinese herbal medicine has been projected to be US 400 billion by 2010 worldwide. Beyond the use as food and supplement, botanical products have been given more serious consideration as complementary and alternative medicine. In June 2004, USFDA issued the "Guidance for Industry Botanical Drug Products" (URL <http://www.fda.gov/cder/guidance/index.html>). Taking certain unique characteristics of botanical drug products into consideration, this guidance makes it non-essential for the sponsor of a botanical drug to identify the active constituents. Instead, FDA requires a combination of tests and controls to ensure the identity, purity, quality, strength, potency, and consistency of botanical drugs. One important objective of these tests and controls is for quality control of botanical raw materials. At the time of the positive development, botanical products are also facing the challenge of product quality and safety. Several incidents which involved botanical products became the targets of negative media attention. In these incidents, wrong herbs were mixed with or used in place of the intended plant species, which resulted in dire consequences.

Quality control of medical herbs is difficult mainly because of the following hurdles:

The lack of clear botanic identity of some traditional medical herbs

The lack of clear botanic identity for herbs is a general phenomenon to many schools of traditional medicine, even for the best-recorded Traditional Chinese Medicine (TCM). Chinese materia medica has had its scope expanded from a handful of drugs recorded around 1000 BC to over 12,800 today. Among the many herbal books by different authors at different times, there were often conflicting records on the identity of herbs under the same generic names. It is also known that people in different geographic regions have used different plant sources under the same generic names. In order to accommodate such traditional use, even the most recent Chinese Pharmacopoeia (2005) still allows some interchangeable use of different herbals under the same generic names. For example, herb Epimedii (Yin Yang Huo) can be any one of the five listed plant species. This practice is acceptable in the context of traditional medicine but creates confusion due to the lack of scientific research on “total equality” and “uniformity”. It also adds to the difficulty in ensuring correct identification and specific use of a particular plant

The arguable use of the term “Dao Di Herbs” or “Authenticated Herbs”

Chinese traditional medicine emphasizes the use of “Dao Di Herbs” or “authenticated herbs,” referring literally to those particular plant species, subspecies or cultivars grown in a particular area and harvested and/or processed in a certain way. However, there is a lack of systematic study on the qualities and criteria of “Dai Di herbs.” There is also no clear and well-accepted definition.

Unknown active principles for many herbs

The active principles of many medicinal herbs remain unknown. As a result, there are many herbs with no quality control at all in terms of chemical composition at all. A practical solution has been adopted for some herbs i.e. checking of marker compounds that are usually the major or specific compounds. Use of marker compounds can be misleading as they might not have direct linkage to bioactivities. Such a practice may also lead to the possibility of adulteration with the specified marker compounds.

Lack of clear bioactivity target for authentication and quality control.

Many herbs have been traditionally used for multiple purposes, presumably due to multiple bioactivities conferred by the various chemical constituents. As an example, Sanqi (, *P. notoginseng*) has used for promoting blood circulation, removal of blood stasis, induction of blood clotting, relief of swelling and alleviation of pain. It was even reported by recent research as having estrogen activity and activity of sensitizing an experimental tumor to ionizing radiation. Many of these bioactivities have been attributed to different saponins. If only one bioactivity or component is used for authentication and quality control, other components and bioactivities are then not taken into consideration.

Other factors that affect safety of herbs

The safety of herbs is affected by intrinsic toxicity of some components. It is also affected by the presence of toxic heavy metals, and agrichemicals such as herbicides/insecticides. Deoxyribonucleic acid (DNA) is the fundamental building components of all living cells. The specific arrangement of DNA base pair sequences guides the production of proteins and enzymes, which in turn will direct the synthesis of a wide range of phytochemicals. DNA profiling, including PCR based techniques and DNA sequencing, can reveal differences in genomic DNAs of living organisms including plants, and their results are generally independent of the environment and developmental stages. Such techniques can distinguish plants from different families, genera and even closely related cultivars. The different techniques vary in setting up terms of cost, throughput, operation cost and reliability. The two most popular techniques are amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR or microsatellite) because of their high level of resolution and robustness. DNA sequencing is also widely used in differentiating herbs at higher taxonomic levels.

Examples of recent research on DNA profiling of medicinal herbs:

- A survey in the Singapore market by AFLP found that some genetically different herbs were labeled and sold under the same generic names.
- SSR, AFLP and Sequencing have been applied to Panax species to define each species and to tell adulterants apart.
- Similarly, DNA profiling has successfully differentiated medical Dendrobium species from other Dendrobium species with no medical use; many species or subspecies of medical herbs have been clearly identified in a similar way such as Hop species, Ephedra species.
- A recent study using both DNA profiling and chemical composition profiling has found genetic and active principle variation in a single farm of Panax notoginseng (Sanqi), suggesting that genetic and chemical composition diversity still exists for such a herb with a long history of cultivation.
- New technology is diffusing into DNA profiling. In a most recent report, an oligo microarray system was developed to detect toxic medical herbs by their specific 5S DNA fragments.

It can be concluded that DNA profiling can clearly identify adulterants, differentiate between herbals of different families, genera, species and even subspecies or cultivars. Genetic identity of a medical herbal can be unambiguously used as proof of genetic uniformity. This will be very useful for proving consistency in plantation and harvest of herbals of the same genetic identity.

However, DNA profiling by itself can prove neither chemical composition nor bioactivities. A solution to this limitation is to adapt a holistic approach by combining the use of DNA profiling with chemical profiling and/or biological activity testing for authentication and quality control of medical herbs. DNA profiling will be more useful if a linkage is created between a genetic profile (or a marker) and the content of the bioactive components and even bioactivities. There are, however, few reports in this area. Because of the uncertainty of the relative contribution of genetic factor and environmental conditions, this is a difficult task. However, DNA profiling is still possible for those herbal species/varieties with strong genetic contribution. It is reported that sequences in the ITS region and chloroplast regions could differentiate Ephedra species with Ephedrine alkaloids from those without. A tight linkage will facilitate wider recognition and adaptation of DNA profiling for quality control of herbals.

Since the process from raw materials to a finished product can take place in different countries, international collaboration in research and development of DNA profiling is necessary and can be conducted in the following areas:

a. Deposit and allow access to voucher specimen:

Voucher specimens are a cornerstone to the whole authentication procedure. They serve as reference material and prove chain of custody. Researchers and manufacturers should be encouraged to deposit sample specimen of herbs that have been properly examined and authenticated to a recognized international herbarium, from which samples are accessible.

b. Deposit and exchange of DNA samples

Together with specimen, DNA samples should also be deposited. DNAs should be made available to others for verification of published results, to compare different protocols and further technical development.

c. Standardization of protocols for DNA profiling

There should be an international effort in working out standard protocols that can be widely adopted. For sequencing based approach, consensus can be reached as to what nuclear and chloroplast genes should be sequenced. More development on technical details is necessary to standardize SSR or AFLP protocols. Information like amplification primers, amplification conditions, equipment and parameters for analysis are all important for others to interpret and repeat experiments. It is not an easy task, but possible, as exemplified by the widely used 13 SSR loci endorsed by the FBI Laboratory's Combined DNA Index System (CODIS) for human typing.

d. Mechanism to share chemical profiling and DNA fingerprinting information

Chemical profiling results and DNA profiles should be deposited and shared, which will clearly be beneficial for the authentication procedure. There is a need for a common platform to present and create database for such information.

In a summary, authentication and quality control of herbal materials have become the key issues to modernization of traditional medicine and promote wider acceptance of herbal products. They are also critical to any research project on herbal medicine to ensure credible research results. Due to the chemical complexity contributed by particular genetic make-up and growth conditions, a holistic and integrated approach should be adopted for quality control purpose. Such an approach should start from certification of herbs by herbalists, followed by DNA profiling, chemical fingerprinting and biological activity testing. More efforts are needed to link DNA profile to chemical components and bioactivities. International collaboration in depositing and accessing voucher specimen, macro- and microscopic data, standardization of DNA profiling protocols for various herbal species, sharing of chemical profiling data and DNA profile information will be very helpful in ensuring quality control of medical herbs and to provide consumers with safe products. 🌱

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Alternative medicine. Quite the same Wikipedia. Just better. Alternative medicine are generally practices that lack biological plausibility and are untested or untestable. In a few cases they are proven ineffective. Complementary medicine (CM), complementary and alternative medicine (CAM), integrated medicine or integrative medicine (IM), and holistic medicine are among many rebrandings of the same phenomenon. What is alternative medicine? In general, the term "alternative therapy" refers to any health treatment not standard in Western medical practice. When used alongside standard medical practices, alternative approaches are referred to as "complementary" medicine. Beyond that, complementary and alternative therapies are difficult to define, largely because the field is so diverse. Chiropractic work is widely accepted in the medical community, and thus qualifies more as a "complementary" medicine than alternative. Alternative medicine is a term that describes medical treatments that are used instead of traditional (mainstream) therapies. Some people also refer to it as "integrative," or "complementary" medicine. More than half of adults in the United States say they use some form of alternative medicine. But exactly what types of therapies are considered alternative? The definition changes as doctors test and move more of them into the mainstream.