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# DNA Barcode Testing in Authentication of Botanical Raw Material Coming of Age

Growing popularity globally in use of botanicals and their processed botanicals (referred as botanicals in the rest of this article), emerging data on their sustainability, availability, and supply chain issues have raised concerns on quality of such products. Botanicals are finding use in health supplements and nutraceuticals, in dermatological and cosmetic preparations, and also in traditional medicines apart from botanical drugs in the US and phytopharmaceuticals in India. Different studies have also pointed out to increase in substitution, adulteration, and use of unauthenticated botanicals. In this context, it is not wrong to state that "the first step of quality for a botanical-based product is to use the botanically authenticated plant and its parts of the correct genus and species."

Botanicals may fall into regulated or unregulated categories based on availability of quality specifications/monographs for them in either individual national pharmacopeias (including pharmacopeia of traditional medicine). Several conventional techniques such as macroscopy, powder microscopy, and other pharmacognostic testing; organoleptic methods; chemotaxonomy; and chemical methods such as thin layer chromatography, high-performance liquid chromatography, fourier transform infrared spectroscopy, and liquid chromatography/mass spectrometry (LC/MS) have their own advantages as well as disadvantages in species authentication and form part of pharmacopoeial monographs. In addition, due to increasing availability of analytical as well as biomarkers and instrumental methods of analysis, heavy dependence on chemical testing is growing. It is to be recognized that there is dearth of trained taxonomists, botanists, and pharmacognosists with competency to authenticate botanical identity. Concurrently shifting of scientific approach to molecular-level studies has led to emergence of DNA barcode testing for botanicals as well for identity.

DNA barcode makes use of short (<1 kb) region of the genome (a barcode) from either nuclear or organelle genome that evolves fast enough to differentiate between closely related species. However, barcoding of plants evolved at much slower pace. It became evident that mitochondrial genome evolves far too slowly in plants to allow it to distinguish between species. A barcode must be flanked by conserved regions that can function as primer-binding site during polymerase chain reaction amplification. An ideal plant barcode needs to be amplifiable with only a single set of primers so that it can be efficiently retrievable from any of the over 200,000 plant species. Thus, a single barcode fulfilling these two requirements has not been found in plants, and a combination of two or more will be required to approach the level of species discrimination and universality. Several gene candidates *matK*, *rbcL*, *trnH-psbA*, *ITS*, *trnL-F*, *5S-rRNA*, and *18S-rRNA* have been tested for use in plants with respect to discrimination capacity. However, it was concluded that no single plant barcode exists. Two international initiatives working toward the development of DNA barcodes include the consortium for the barcode of life (CBOL) and international barcode of life project, after several consultations with stakeholders, and evaluating seven chloroplast genomic regions across plant kingdom proposed the use of *matK-rbcL* combination as a potential barcode for land plants, but with an option to supplement it with one or two other markers, *psbA-trnH* or *ITS*. A combination of these two can help achieve maximum species discrimination. Later, China Plant BOL Group proposed addition of nuclear *ITS* to the *matK-rbcL* combination as barcode to achieve maximum identification rates even in closely related species. *ITS* is by far the most widely sequenced locus for angiosperms compared to 30,325 entries for *rbcL*, which is most frequently sequenced plastid gene. Obviously, this should make it most suitable barcoding region if quick identifications are desired. On the other hand, *ITS* is discredited due to its nonlinear pattern of evolution in some groups of plants. However, the presence of universal primer for the ITS region and its evolutionary divergence rate suggests that its use as barcode should perhaps be considered, not discrediting entirely. Review of status on developing bioinformatics tools and resources to support barcoding of all organisms on the planet is available. The Barcode of Life Data System (BOLD; http://www.barcodinglife.org/views/login.php) was the result of such efforts made by CBOL to facilitate easy deposition and retrieval of data on barcodes. BOLD provides an integrated bioinformatics platform for all phases of the analytical pathway from specimen collection to tightly validated barcode library. It generates varied distance matrix to construct a neighbor-joining tree labeling the terminal branches with taxonomic information, locality data, and/or sequence length. Unknown specimens in the samples can also be identified using BOLD. The query sequence is aligned quickly to the global alignment through the hidden Markov model followed by a linear search of the reference library. In this way, it tries to identify the possible species. If the species-level search fails, it tries to search possible genus or higher levels. It should be kept in mind that to submit a sequence as "barcode," it must be derived from a gene or genome region that is accepted by CBOL. NCBI has provided a web-based barcode submission tool (BarSTool) for submitting sequences of barcodes.

A number of researchers have studied and published DNA barcode data of raw botanicals, substitutes, and adulterants available in the market in *ITS2* region, and these studies have concluded that barcode can successfully be used to differentiate the species and adulterants. Combining barcode data with nuclear magnetic resonance (NMR) have been shown to clearly determine adulteration, for example, in most often used Indian plant for treating women's disorders, namely *Saraca asoca*. It is not intended to cover all those studies in this editorial but to state that DNA barcode testing studies have covered botanicals used in India, China, and Western nations. Studies also point serious concerns on quality in botanical-based supplements, nutraceuticals, and herbal teas.

United States Pharmacopoeia, British Pharmacopoeia, and Indian Pharmacopoeia have in recognition of this technique included general monographs providing detailed guidance and test methods for using either barcode in *ITS* or other regions or testing for intact nucleic acid base. Indian Pharmacopoeia has even prescribed a DNA barcode test as a final alternative when other tests for identity fail for *Asparagus racemosus* (Shatavari). However, DNA testing cannot be a final answer as it has its limitations in detecting authenticity of processed products and finished formulations due to degradation of DNA fragments. At the same time, DNA barcode is incapable of identifying chemical constituents or plant parts or quantify of plant material used in the product. In such cases, DNA barcode authentication has to be supplemented with NMR, LC-MS, or even metabolomics. Validated test methods to extract DNA from varying matrix of products, *viz.*, food matrix, pharmaceutical matrix, and cosmetic matrix, are not available and pose its own challenges basis proportion of the botanical in the matrix. In addition, many of the bioinformatic databases which were freeware are becoming chargeable adding to the cost of testing. As per authors analyses many

buyers in the USA are demanding authentication certificates of the botanicals using DNA barcode technique as part of their purchase requirements. Regulators may enhance dependence on barcode testing in the monographs, to build confidence among patients and consumers.

Pharmacognosists need to learn and build competency in this area so that their role continues to be relevant and contribute to the growing sector. Further, authors propose that the manufacturers of products, dietary supplements, and extracts may develop DNA mini-barcodes of shorter than 300 bp to test finished formulations and dietary supplements and validate before being made available to buyers. Thus, we can create confidence among buyers with regard to authenticity of botanical raw material used. The ultimate aim is to provide safe products with label claim potential delivered to the consumer, thus creating positive image of botanical industry.

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