

# PhytoAuthent: Molecular authentication of complex herbal food supplements for safety and efficacy

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## Abstract

The PhytoAuthent project was structured to gather, test, develop and apply, in real life case scenarios, molecular techniques, such as biochemical fingerprinting and DNA sequence-based methods, for plant identification of constituents in complex herbal products. The project had a strong focus on applied aspects like protecting consumers from health risks associated with product substitution and contamination of herbal products.

## Keywords

authentication, DNA barcoding, DNA metabarcoding, herbal products, medicinal plants

## Introduction

The [European Medicines Agency \(EMA\)](#) is the sole responsible body for the evaluation of medicinal products in Europe, but with limited attributions related to herbal products because it lacks the regulations and the tools to uniformly monitor and control herbal products. The [EMA](#) supports the use of innovative analytical technologies, such as DNA barcoding, to complement traditional chromatographic identification methods for substitute, filler and adulterant detection in herbal products. Many herbal preparations and products have a history of safe use, but there is growing concern about their quality, efficacy and safety, as a result of studies showing major discrepancies between label information and analysed composition (Raclariu et al. 2018a).

The [PhytoAuthent project](#) successfully addressed the ambiguity issues of the authenticity of herbal products (Fig. 1), with possible negative effects on the efficiency and safety of these products and, implicitly, on consumer's well-being and health (de Boer et al. 2015). Around 200 different herbal products were bought from across the Europe and introduced in our study. The focus was on adapting and testing the feasibility of using high-throughput DNA sequencing (HTS) methods, which use the DNA sequence barcode concept as a quick and accurate way to identify up to species level using standard DNA regions.

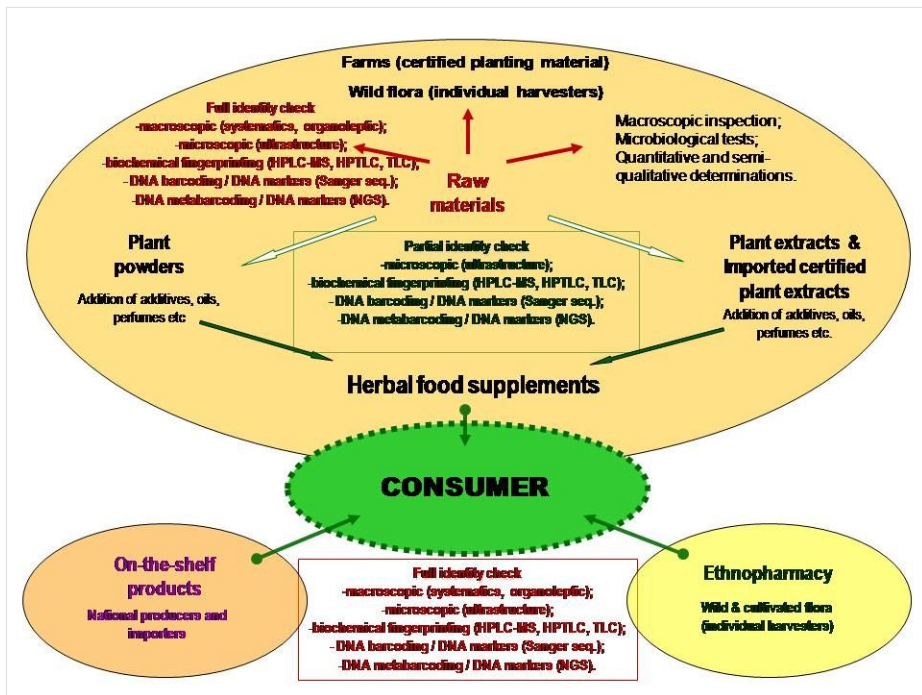


Figure 1. [doi](#)

Value chains of herbal food supplements studied by the PhytoAuthent project.

## Description

DNA metabarcoding was used to authenticate herbal products containing *Echinacea* (Raclariu et al. 2018b), *Hypericum* (Raclariu et al. 2017a) and *Veronica* (Raclariu et al. 2017a) DNA metabarcoding of 78 herbal products containing *Hypericum perforatum* L. (St. John's wort) detected the target species in only 68% of products (Raclariu et al. 2017b). Authentication of 16 herbal products containing *Veronica officinalis* L. (common speedwell) though DNA metabarcoding detected the target species in only 15% of the products, but detected a related species, *V. chamaedrys* (germander speedwell) as an undeclared ingredient in 62% of the products (Raclariu et al. 2017a). Three common species of *Echinacea* were targeted in 53 herbal products and using DNA metabarcoding, *Echinacea* species were detected in 34 out of the 38 retained products (89%), but with a lack of discriminatory resolution at the species level (Raclariu et al. 2018b). It should be noted that DNA barcoding and metabarcoding detected inconsistency between reported constituents and identified species in all analysed products.

In all our studies we focused on comparative methodological studies, where we compared DNA-based methods with biochemical analysis methods recommended by different pharmacopoeias for quality assessment, such as High Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS), High Performance Thin Layer Chromatography (HPTLC) and Thin Layer Chromatography (TLC). These biochemical fingerprinting methods, which have proved to be exact methods for authentication of the presence of target bioactive compounds of the analysed species, had limited effectiveness in the detection of substitution with related plant species and could not provide any information about other unknown ingredients of plant origin from the products.

## Conclusions

If the safety of the herbal products is interpreted to the limit of specific bioactive compounds, then methods of chemical analysis are more relevant than analysis of the composition based on the DNA sequence data, but when analysing product authenticity, species substitution or falsification, DNA barcoding and DNA metabarcoding offer incomparable resolution in species diversity detection (Raclariu et al. 2018a).

## Recommendations

Our results support the use of DNA based methods (DNA barcoding and metabarcoding) for the authentication of complex herbal products. The adoption of quality control standards by regulatory and control bodies could increase product quality and subsequently increase consumer confidence. Such standards would provide also an incentive for producers to increase internal quality control throughout the whole production chain, from plant raw materials and extracts to the final product.

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## Hosting institution

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## Author contributions

MCI initiated the project. MCI, GC, CET and HdB designed the experiments and supervised their teams as Project Director (MCI) and Principal Investigators (GC, CET and HdB). MCI wrote the initial draft of the manuscript. MCI, GC, CET and HdB revised and reviewed the manuscript.

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