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Successful isolation and PCR amplification of DNA from National Institute of Standards and Technology (NIST) herbal dietary supplement standard reference material powders and extracts

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Abstract

Twenty-four herbal dietary supplement powder and extract reference standards provided by the National Institute of Standards and Technology (NIST) were investigated using three different commercially available DNA extraction kits to evaluate DNA availability for downstream nucleotide-based applications. The material included samples of *Camellia*, *Citrus*, *Ephedra*, *Ginkgo*, *Hypericum*, *Serenoa*, and *Vaccinium*. Protocols from Qiagen, MoBio, and Phytopure were used to isolate and purify DNA from the NIST standards. The resulting DNA concentration was quantified using SYBR Green fluorometry. Each of the twenty-four samples yielded DNA, though the concentration of DNA from each approach was notably different. The Phytopure method consistently yielded more DNA. The average yield ratio was 22:3:1 (ng/μL; Phytopure:Qiagen:MoBio). Amplification of the Internal Transcribed Spacer II region using PCR was ultimately successful in twenty-two of the twenty-four samples. Direct sequencing chromatograms of the amplified material suggested most of the samples were comprised of mixtures. However, the sequencing chromatograms of twelve of the twenty-four samples were sufficient to confirm the identity of the target material. The successful extraction, amplification, and sequencing of DNA from these herbal dietary supplement extracts and powders supports a continued effort to explore nucleotide sequence-based tools for the authentication and identification of plants in dietary supplements.

Keywords

herbal dietary supplement; DNA

Herbal dietary supplements comprise a large over-the-counter global market and generally consist of raw shredded or powdered vegetative material or extracts of vegetative material. Preparations of herbal dietary supplements may consist of one or more species depending on the formulation. Identity testing of plants contained in herbal dietary supplements as a means to ensure product quality and safety is an ongoing concern for government regulators, commercial suppliers, trade groups, and researchers [1,2]. Traditional means of identification using morphological characters are not practical when the target material occurs as a mixture or a single element in powdered or extracted forms [3]. The predominant alternative using biochemical profiles is also problematic due the temporal, spatial, and genetic differences of plant populations that directly impact the production of secondary metabolites [4-6]. The use of nucleotide sequence data offers a means of identity testing that is independent of sample form, concentration, and purity, as well as when and where plant tissues were collected [7,8]. Of course, the utility of nucleotide sequence data for identification depends on the availability

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of DNA in processed forms of botanical material associated with herbal dietary supplements. This paper reports the successful isolation, PCR amplification, and direct sequencing of DNA from herbal powders and extracts supplied by the National Institute of Standards and Technology, including samples of *Camellia*, *Citrus*, *Ephedra*, *Ginkgo*, *Hypericum*, *Serenoa*, and *Vaccinium* (see Table 1).

Three commercially available DNA purification kits were evaluated for their ability to effectively isolate DNA from samples of herbal dietary supplement pastes and extracts, including Qiagen DNeasy (Valencia, CA), MoBio PowerClean (Carlsbad, CA), and GE Healthcare Phytopure (Buckinghamshire, UK). The protocols were executed according to the manufacturer's guidelines using 16-18 mg of processed botanical sample material in each extraction. The DNA yield from each protocol was measured using a Turner BioSystems fluorometer (TBS-380, Sunnyvale, CA) with SYBR green dye (Carlsbad, CA) and 5 μ L of extraction product from each sample. The recorded values (ng/ μ L) for the extraction DNA concentration are presented in Table I.

The selected DNA extraction protocols isolate DNA using two common mechanisms, organic separation followed by precipitation (Phytopure) and silica membrane spin columns (Qiagen and MoBio). Each of the selected methods successfully isolated DNA from the herbal dietary supplement samples. However, the concentration of DNA from each approach was notably different. The Phytopure method consistently yielded more DNA product. The average yield concentration ratio was 22:3:1 (Phytopure:Qiagen:MoBio).

The final concentration of DNA from each of the extraction protocols is not sufficient to demonstrate that the extraction products can be used in downstream applications, especially those using PCR. Approximately 2.5ng to 25ng of DNA from each extraction product was used in PCR along with primers targeting the nuclear encoded Internal Transcribed Spacer II region [9]. The names and sequences for these primers are ITS3 (forward): GCATCGATGAAGAACGCAGC and ITS4 (reverse): TCCTCCGCTTATTGATATGC. The initial round of PCR was partially successful using DNA from each method. Ten samples amplified using DNA from the Qiagen method, two the Phytopure method, and two from the MoBio method. Continued PCR effort with the extraction products obtained using the Qiagen method and nested primers for the Internal Transcribed Spacer region (i.e. forward primers ITS5 and ITS3 with reverse primer ITS4; [9]) produced amplified product for twenty-two of the samples used in the study. The sequence for the ITS5 forward primer used in this study is GGAAGTAAAAGTCGTAACAAGG. Direct sequencing was performed on the amplified product from the Qiagen extracted samples and the resulting data were taxonomically characterized to the genus level using the Basic Local Alignment Search Tool (BLAST; [10]. Examination of the sequencing chromatograms suggested that most of the samples were comprised of mixtures. However, twelve of the twenty-four samples did sequence well enough to confirm the identity of the target material or another botanical component of the reference sample (see Table 2).

This project evaluated DNA availability in a set of NIST herbal dietary supplement standard reference material powders and extracts, forms of botanical material that are common in the retail supply chain. Extraction of DNA was achieved using three different commercially available kits and the success of each method was ranked by the quantity of DNA isolated from each sample, as well as the downstream success of PCR amplification using the isolated and purified DNA. In this study, the Qiagen DNeasy extraction kit produced the highest number of DNA samples (22 of 24) that could be amplified in PCR. This work suggests that DNA can be routinely extracted from common forms of herbal dietary supplement extracts and powders, supporting a continued effort to explore DNA-based methods for quality assurance and quality control of herbal dietary supplements.

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Abbreviations

BLAST	basic local alignment search tool
DNA	deoxyribonucleic acid
NIST	National Institute of Standards and Technology
PCR	polymerase chain reaction

Table 1

List of herbal dietary supplement samples and the corresponding yield of isolated DNA (ng/μL) using each of the specified commercially available extraction methods.

Sample#	Description	Qiagen	Phytopure	MoBio
1	<i>Camellia sinensis</i> Extract (Green tea)	3.8	4.7	0.2
2	<i>Camellia sinensis</i> Leaves (Green tea)	10.1	61.6	7.9
3	<i>Camellia sinensis</i> -Containing Solid Oral Form (Green tea)	7.5	59.1	0.7
4	<i>Citrus aurantium</i> Bitter Orange Extract	22.2	271.8	10.3
5	<i>Citrus aurantium</i> Bitter Orange Fruit	8.7	196.0	2.9
6	<i>Citrus aurantium</i> -Containing Solid Oral Form	6.1	121.4	2.1
7	<i>Ephedra sinica</i> Aerial Parts (Mormon tea)	10.4	105.2	8.0
8	<i>Ephedra sinica</i> Aerial Parts (Mormon tea)	10.9	90.9	7.0
9	<i>Ephedra sinica</i> Commercial Extract (Mormon tea)	10.7	170.5	5.1
10	<i>Ephedra sinica</i> Native Extract (Mormon tea)	6.5	298.1	8.2
11	<i>Ephedra sinica</i> -Containing Solid Oral Form	11.2	71.0	3.5
12	<i>Ginkgo biloba</i> Extract	1.6	3.0	0.5
13	<i>Ginkgo biloba</i> Leaves	66.8	88.9	8.6
14	<i>Ginkgo biloba</i> Leaves	68.0	91.3	8.0
15	<i>Ginkgo biloba</i> -Containing Tablets	0.7	2.3	0.5
16	<i>Hypericum</i> Methanol Extract (St. John's Wort)	1.0	3.8	0.7
17	<i>Hypericum</i> Solid Oral Dosage Form (St. John's Wort)	1.6	9.6	0.8
18	<i>Serenoa repens</i> Fruit Extract (Saw palmetto)	8.1	44.9	0.8
19	<i>Vaccinium corymbosum</i> (Blueberries)	8.3	49.6	3.7
20	<i>Vaccinium macrocarpon</i> (Cranberries)	2.8	44.2	1.3
21	<i>Vaccinium macrocarpon</i> (Cranberry) Extract	0.9	25.4	1.0
22	<i>Vaccinium macrocarpon</i> (Cranberry) Extract	1.1	23.4	1.3
23	<i>Vaccinium macrocarpon</i> -Containing Solid Oral Form	1.4	20.8	1.0
24	<i>Vaccinium spp.</i> -Containing Solid Oral Form	4.8	53.9	0.6
	Average Yield by Extraction Method	11.5	79.6	3.5

Table 2

Generic level taxonomic characterization of DNA sequence data determined from NIST standard reference material of herbal dietary supplement powders and extracts.

Sample #	Description	Taxonomic Characterization
1	<i>Camellia sinensis</i> Extract (Green tea)	No PCR Product
2	<i>Camellia sinensis</i> Leaves (Green tea)	Mixture
3	<i>Camellia sinensis</i> -Containing Solid Oral Form (Green tea)	<i>Camellia</i>
4	<i>Citrus aurantium</i> Bitter Orange Extract	No PCR Product
5	<i>Citrus aurantium</i> Bitter Orange Fruit	<i>Citrus</i>
6	<i>Citrus aurantium</i> -Containing Solid Oral Form	Mixture
7	<i>Ephedra sinica</i> Aerial Parts (Mormon tea)	<i>Ephedra</i>
8	<i>Ephedra sinica</i> Aerial Parts (Mormon tea)	<i>Ephedra</i>
9	<i>Ephedra sinica</i> Commercial Extract (Mormon tea)	<i>Ephedra</i>
10	<i>Ephedra sinica</i> Native Extract (Mormon tea)	<i>Ephedra</i>
11	<i>Ephedra sinica</i> -Containing Solid Oral Form	<i>Ephedra</i>
12	<i>Ginkgo biloba</i> Extract	Mixture
13	<i>Ginkgo biloba</i> Leaves	Mixture
14	<i>Ginkgo biloba</i> Leaves	Mixture
15	<i>Ginkgo biloba</i> -Containing Tablets	Mixture
16	<i>Hypericum</i> Methanol Extract (St. John's Wort)	Mixture
17	<i>Hypericum</i> Solid Oral Dosage Form (St. John's Wort)	Mixture
18	<i>Serenoa repens</i> Fruit Extract (Saw palmetto)	Mixture
19	<i>Vaccinium corymbosum</i> (Blueberries)	<i>Vaccinium</i>
20	<i>Vaccinium macrocarpon</i> (Cranberries)	<i>Vaccinium</i>
21	<i>Vaccinium macrocarpon</i> (Cranberry) Extract	Mixture
22	<i>Vaccinium macrocarpon</i> (Cranberry) Extract	Mixture
23	<i>Vaccinium macrocarpon</i> -Containing Solid Oral Form	<i>Vaccinium</i>
24	<i>Vaccinium spp.</i> -Containing Solid Oral Form	<i>Vaccinium</i>