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DNA Barcoding of Herbal Products to Determine Mislabeling on the U.S. Commercial Market

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Introduction

Herbal Product Mislabeling

The international trade in herbal products is a huge market. The demand for herbal products has increased over the last few years and it is continuing to increase. Today, there are more than 1,000 companies producing medicinal plant products. These companies get an annual revenue of about US$60 billion (Newmaster, 2013). Herbal supplements may be contaminated or substituted with alternative plant species and fillers that are not listed on the labels. Federal law does not require the manufacturer or seller to prove to FDA's satisfaction that the supplements are safe or that the claims are accurate or truthful before appearing on the market. This can result in a threat to public safety.

Figure 1. Herbal Supplements tested in this study. Pictured: Amla, ginger, and tulsi.

DNA Barcoding

Product mislabeling occurs when a food product's label does not accurately reflect its ingredients. In this study, three herbal categories were tested. The method that was used to test these products was DNA barcoding. DNA barcoding is a species identification tool that targets short DNA sequences and compares them to DNA sequence data bases (Newmaster et al. 2013).

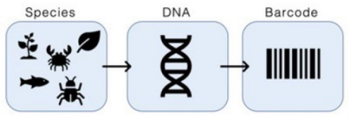

Figure 2. The process of DNA barcoding. A short, standardized sequence of DNA is used as a unique barcode for species identification. (Agri-Pulse)

Objective

The objective of this project was to investigate species substitution mislabeling of amla, ginger, and Tulsi herbal products on the U.S. commercial market.

Materials & Methods

Sample Collection

- A total 3 different herbal categories were chosen for sampling: *Emblica officinalis* (amla), *Zingiber officinale* (ginger), and *Ocimum sanctum* (tulsi). Samples were labeled as Alma (n= K06, K08, K12, K13, K20), Ginger (n= K04, K10, K15, K18, K22), Tulsi (n= K14, K17)
- The samples were obtained from online sources and supplement stores.
- Samples were catalogued, then 4-5 five capsules were mixed to make a composite sample. The samples were stored in a labeled 50 mL Falcon Tube at room temperature.
- 100 mg of each sample was placed in a labeled 1.5 mL microcentrifuge tube for extraction.

Materials & Methods (cont'd)

DNA Extraction

- DNA extraction for this experiment used the NucleoSpin Plant II kit (Macherey-Nagel) . This extraction kit was used on all the samples that were tested. (Fig. 3)
- A reagent blank with no sample DNA was included for every set of extractions.
- All extracted DNA was stored at 4°C until the next process of amplification with the polymerase chain reaction (PCR) or, for long-term storage, placed in the -20 °C freezer.

Figure 3. DNA extraction method (image credit: Bioke)

PCR and DNA sequencing

- DNA Barcoding was carried out on all samples using the protocols described in Fazekas et al (2012)
  - PCR targeted regions of rbcL and ITS2. 2X Platinum Hot Start PCR Master Mix was used with each of the samples.
  - A non-template control with water instead of DNA was included for each set of samples that were tested.
- Gel electrophoresis was used to confirm amplification. The samples were placed in 2% agarose E-gels (Invitrogen).
  - E-Gel 1 kb Plus DNA Ladder (Invitrogen) was used.
- PCR products were stored at –20°C and cleaned with ExoSAP-IT (Affymetrix)
- PCR products were sequenced by Eurofins Genomics.

Figure 4. Thermal Cycler used for PCR (Eppendorf)

Figure 6. 2% agarose E-Gel with sample bands from PCR product

Results & Discussion

Table 1 shows the bands that were present when the samples underwent gel electrophoresis. When using the ITS2 primer, the alma samples had 1 band present out of the 5 samples that were tested. For the ginger samples, there were bands for all the samples. The tulsi samples had 2 bands out of the 2 samples tested. When the rbcL primer was used, the alma samples had 4 out of 5 samples that showed a band, the ginger samples showed 5 out of 5 bands, and the tulsi samples had 2 bands out of the 2 samples tested.

Table 1. The number of bands obtained with gel electrophoresis from each sample category post-PCR

Sample Number	Sample Category	# of Bands with ITS2	# of Bands with rbcL
K06, K08, K12, K13, K20	*Emblica officinalis* (amla)	1	4
K04, K10, K15, K18, K22	*Zingiber officinale* (ginger)	5	5
K14, K17	*Ocimum sanctum* (tulsi)	2	2

Results & Discussion (cont'd)

Table 2 shows the sequencing results for the samples. None of the samples showed sequencing data with ITS2. There was more success when using the rbcL. The overall mislabeling rate among samples with sequencing data was 1/8 (12.5%). None of the amla samples showed successful sequencing results. When the ginger samples were tested, 80% (4/ 5) of the samples tested matched the DNA sequence for ginger. The remaining sample did not have ginger detected. One sample labeled as containing tulsi was identified as Wild cassia/Matura tea tree (*Senna* sp.) while another was identified as containing the expected species (*Ocimum* sp.).

Table 2. Preliminary* sequencing results for herbal samples

Sample label	# of products with sequences	# of products declared species detected	# Potentially mislabeled	Undeclared species detected with rbcL	Undeclared species detected with ITS2
Amla	0	N/A	N/A	N/A	N/A
Ginger	4	4	N/A	N/A	N/A
Tulsi	2	1	1	Wild cassia/Matura tea tree (*Senna* sp.)	N/A

*Additional testing will be carried out to confirm these initial results and to optimize recovery of plant barcodes from the remaining samples.

Conclusions

The mislabeling of herbal products is a growing issue as there is an increase in consumption. The samples ginger, amla, and tulsi were used for testing as they are very popular in the market. Product mislabeling is a public safety matter as consumers may not know the actual ingredients that are in the product. Once the samples were sent for sequencing, the samples were able to be analyzed and compared with the DNA of the focused herb (i.e., ginger, amla, tulsi). For the first set of data with the ITS2 primer, it was difficult to make a clear conclusion due to the ambiguities in the samples. There proved to be more success in DNA sequencing when using the rbcL primer. With further research and sample collection, we can work towards identifying the contents of the product and demonstrate a need for increased scrutiny into the contents of herbal supplements.

References

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I worked in Dr.Hellberg's Food Science Protection Lab this summer where we investigated species mislabeling of amla, ginger, and Tulsi herbal products on the U.S. commercial market. The method we used was DNA Barcoding in order to test the samples.

Alternate Text:

Miranda Miranda

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Image of Miranda Miranda

Image of text and graphic laden project presentation entitled "DNA Barcoding of Herbal Products to Determine Mislabeling on the U.S. Commercial Market. M. Miranda, C. Harris, C. Jordan, R.S. Hellberg"