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File: ■ Saw Palmetto (*Serenoa repens*, Arecaceae)
■ DNA Barcode Authentication
■ Misidentification

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RE: Saw Palmetto DNA Mini-barcode Assay Developed to Distinguish between Authentic and Spurious Material in Dietary Supplements

Little DP, Jeanson ML. DNA barcode authentication of saw palmetto herbal dietary supplements. *Sci Rep*. December 17, 2013;3:3518. doi: 10.1038/srep03518.

Saw palmetto (*Serenoa repens*, Arecaceae) grows in the southeastern United States and produces edible fruits. Native American people used saw palmetto traditionally for the treatment of gastrointestinal problems and as a diuretic.¹ Saw palmetto is currently used in the treatment of benign prostate hyperplasia; clinical trials suggest its efficacy and safety.¹ It is essential that botanicals used as dietary supplements be definitively identified. DNA barcoding is one way to confirm species identification. The genes *matK* and *rbcL* encode plastid proteins and are commonly used for DNA barcoding in plants. This basic research study set out to produce a DNA barcode library for the identification of saw palmetto, introduce an assay based on a DNA barcode for saw palmetto identification, and test this assay on saw palmetto supplements commercially available in the United States. Plant parts in supplements were not described beyond being "dry, cut, and sifted plant materials."

The *matK* (n=27) and *rbcL* (n=37) barcode sequences were generated from dried leaves of 37 identified specimens from several species. A distinction between saw palmetto and the related species in the authenticated leaf material was achieved by a combination in nucleotide differences in the full-length *matK* and *rbcL* sequences. However, amplification of full-length barcode sequences in the commercial dietary supplements using polymerase chain reaction (PCR) did not work in this study due to fragmented DNA. To circumvent this problem, the authors composed mini-barcode primers to amplify specific nucleotide ranges of *matK* and *rbcL* that could distinguish saw palmetto from other species. The mini-barcodes were validated using the authentic leaf specimens and tested on saw palmetto supplements. For validation, 13 samples of saw palmetto and 18 samples of a closely related species to saw palmetto, the Paurotis palm (Everglades palm; *Acoelorrhaphe wrightii*, Arecaceae), were analyzed. All validation samples were correctly identified using the combination of *matK* and *rbcL* mini-barcodes. Specificity and sensitivity were both 1.00, with 95% confidence intervals of 0.74-1.00 and 0.66-1.00, respectively.

From a total of 37 saw palmetto supplements, 34 yielded amplifiable DNA. In 30 of the supplements, both *matK* and *rbcL* mini-barcodes were successfully sequenced. In total, 29 supplements definitely contained saw palmetto; the mini-barcode of *rbcL* was unobtainable in three other supplements. Since the *rbcL* sequence allows distinction between saw palmetto and the Paurotis palm, the authors were unable to determine whether they had saw palmetto or Paurotis palm or both in these three products. Of the 34 supplements where DNA could be obtained, two were found to be adulterated; one product contained Paurotis palm, and one had another unidentifiable species.

In conclusion, the authors state that initial failure of PCR in obtaining barcodes from dietary supplements is not unusual, especially when handling longer regions of DNA. To get around this, the mini-barcodes were devised and validated for identification of both specimens and supplements of saw palmetto. It is speculated that the reasons for failing to obtain DNA from three of the dietary supplements may be due to absence of saw palmetto itself, degradation of DNA due to the processing, or inhibition of sequencing during PCR. It is also surmised that the *rbcL* mini-barcode may be more sensitive to degradation than that of *matK*.

Despite these technical limitations, if DNA from both mini-barcode sequences is obtained, distinctive identification of saw palmetto from even its closely related species, the Paurotis palm, is definitive. It is concluded that conflicting results in clinical studies involving saw palmetto supplements may be due, at least in part, to the use of adulterated products. This study provides a robust method by which to determine the purity of saw palmetto supplements, and it can be used to identify contamination of supplements by additional species.

—Amy C. Keller, PhD

Reference

¹Blumenthal M, Goldberg A, Brinckmann J, eds. *Herbal Medicine: Expanded Commission E Monographs*. Austin, TX: American Botanical Council; Newton, MA: Integrative Medicine Communications; 2000.

Referenced article can be accessed at
<http://www.nature.com/srep/2013/131217/srep03518/full/srep03518.html>.

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