Uncovering bark: The use of DNA barcoding to identify unknown bark species illegally traded at the Faraday 'Muthi' Market in Johannesburg

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Abstract

The illegal trade of bark from indigenous plant species is a growing trend that goes hand in hand with traditional belief systems in South Africa. Certain barks are thought to have medicinal and magical properties, and are widely used as an alternative to Western medicines. The growing demand for traditional medicines has inevitably led to unsustainable and careless harvesting practices by traditional healers and collectors who are often unaware of environmental laws and regulations. It has also led to some species being adulterated or substituted with others, which can pose serious health risks. Furthermore, the barks are often processed in such a way that identification by conventional methods are impossible. Here we employed DNA barcoding to authenticate bark and bark derived products sold at the Faraday traditional medicinal market in South Africa. **Results.** The current reference data set comprises over 1400 woody plant species of southern Africa, which represent 66% of the approximately 2200 species (115 families and 541 genera) in the region. This data set was used to identify and assign species names to bark samples and their derivatives obtained from the market. **Significance**. This study highlights threatened species found at the market. Furthermore, the study also aimed to implement viable, educational solutions that will focus on environmental laws and the importance of biodiversity in the country.

(a)

INTRODUCTION

Traditional medicinal practises has a long standing and rich history in South Africa and is preferred over modern alternatives for being an affordable and accessible substitute. This practise has, however, exacerbated the illegal trade of indigenous plant species.



RESULTS, DISCUSSION & CONCLUSION



Figure 1: Faraday 'Muthi' Market in Johannesburg, South Africa. Picture credit: www.2summers.net.

Some traditional healers and collectors are often unaware of environmental laws and regulations and consequently leads to careless harvesting. Of the many plant parts harvested, barks are thought to have medicinal and magical properties and as such is a popular sight at traditional 'Muthi' Markets.

The barks are often processed in such a way that identification by conventional methods are impractical. Therefore DNA barcoding has been incorporated into identifying species that could be potentially threatened.

Aims of the study

- To identify unknown bark specimens up to species level using DNA barcoding.
- To corroborate the vernacular names that were assigned to species by Williams (2000).
- To expand upon the reference data.

MATERIAL & METHODS

Market Sampling

The plants were collected from Faraday 'Muthi' Market,

Figure 2: (a) Variety of forest products at Faraday 'Muthi' Market, (b) *Prunus africana* with stripped bark

Of the 24 bark query samples collected during this study, 19 could be identified up to species level using DNA barcoding. Only 10 of the query samples collected using their vernacular names, matched with the species identified by Williams (2000). This is most likely due to different species having the same vernacular name. Furthermore, a number of species could have been adulterated or substituted. This is quite concerning as it could pose serious health risks.

Table 1: List of bark samples with their vernacular name and barcoded scientific name

| Scientific name | Vernacular name | Family | Barcoded scientific name | Plant Identification (%) <i>rbcLa</i> | Plant Identification (%) <i>matK</i> |
|--|--------------------------|---------------------------------|--|---|--|
| Albizia adianthifolia (Schum.) W.Wight | mgadankawu | Luguminosae | <i>Albizia odoratissima</i> (L.f.) Benth. | 99% | 98% |
| | C . | C C | · · · · | 96% | |
| Balanites maughamii Sprague Bersama lucens (Hochst.) Szyszył. | ipamba isindiyandiya | Zygophyllaceae Melianthaceae | Balanites maughamii Bersama lucens | 96% | N/A 89% |
| | memezobovu | Rhizophoroceae | Cassipourea sericea (Engl.) Alston | 100% | 99% |
| Cassipourea flanaganii (Schinz) Alston | | * | 1 (2) | | |
| Cinnamomum camphora (L.) J.Presl | Rosaline | Lauraceae | Cinnamomum camphora | 100% | 100% |
| Croton sylvaticus Hochst. | dumbadlozi | Gentianaceae | Anthocleista grandiflora Gilg | 100% | 99% |
| Cryptocarya myrtifolia Stapf | mkhondweni ilukuluku | Lauracea Sterculiacea | Cryptocarya densiflora Blume | 100% | 90% 99% |
| Dombeya rotundifolia (Hochst.) Planch. | | | Dombeya sp. | | |
| Erythrophleum lasianthum Corbishley | mkhwangu | Leguminosae | Erythrophleum ivorense A.Chev. | 100% | 100% |
| <i>Eucalyptus</i> sp. | mdlavuza | Myrtaceae | <i>Eucalyptus</i> sp. | 100% | 100% |
| Garcinia livingstonei T.Anderson | isaphulo | Euphorbiaceae | Croton gratissimus Burch. | 99% | 98% |
| Macaranga capensis (Baill.) Sim | phumelelo | Euphorbiaceae | Macaranga capensis | 95% | N/A |
| Maytenus undata (Thunb.) Blakelock | dabulovalo | Celastraceae | Maytenus undata | 100% | 99% |
| Ocotea bullata (Burch.) E. Meyer in Drege | nukani | Lauraceae | Ocotea bullata | 100% | 100% |
| Olinia radiata J. Hofmeyr & Phill. | mzaneno | Oliniaceae | Erthrococca sp. | 94% | 97% |
| Pinus sp. | abaphaphi | Olacaceae | Heisteria parvifolia Sm. | 100% | N/A |
| Pittosporum viridiflorum Sims | mfusamvu | Pittosporaceae | Pittosporum undulatum Vent. | 100% | 99% |
| <i>Podocarpus henkelii</i> Stapf ex Dallim. & B.D.Jacks. | amanqonqoza | Araucariaceae | Araucaria schmidii de Laub. | 98% | N/A |
| <i>Prunus africana</i> (Hook.f.) Kalkman | Nyazangoma- elimnyama | Rosaceae | Prunus africana | 100% | N/A |
| Pterocelastrus rostratus Walp. | sehlulamanye | Celastraceae | N/A | 100% | N/A |
| Pterocelastrus tricuspidatus Walp. | suhlulamanye | Celastraceae | Pterocelastrus tricuspidatus | 100% | 99% |
| Sclerocarya birrea (A.Rich.) Hochst. | mganu | Anacardiaceae | Sclerocarya birrea | 100% | 99% |
| Turraea floribunda Hochst. | madlozana | Meliaceae | <i>Turraea sericea</i> Sm. | 100% | N/A |
| Vachellia xanthophloea (Benth.) P.J.H. Hurter | mkhanyakude | Leguminosae | Vachellia tortilis (Forssk.) Galasso & Banfi | 100% | 100% |

The status criteria of each species was determined using the SANBI Red List. Figure 3 shows a bar graph of the different status criteria of each species.

sampled and catalogued at the ACDB lab (Figures 1 & 2). The traders were questioned about the medicinal use and vernacular names of the plants.

DNA Barcoding

DNA was extracted from bark material using the 10X CTAB method (Doyle and Doyle, 1987). Standard DNA barcoding protocols were used as set out by the CBOL Plant Working Group (2009).

Species identification

Unknown bark samples were assigned to species using BLAST to compare sequences on GenBank and BOLD. Tree-based identification was also used to verify the obtained BLAST results.

Data analysis

Vernacular names of identified species were checked to see if they corroborated with those recorded by Williams (2000). The SANBI Red list of endangered species was then used to check the conservation status of all query samples.

ACKNOWLEDGEMENTS



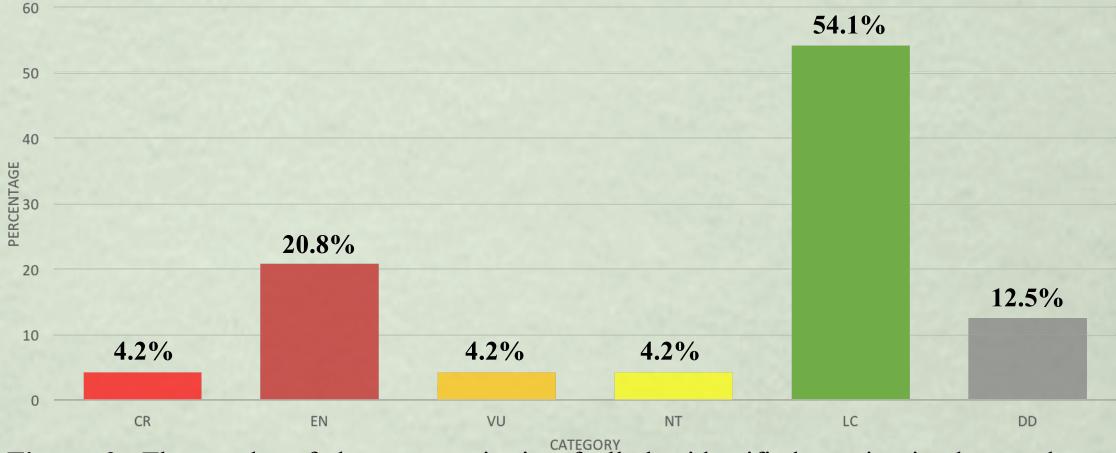


Figure 3: The results of the status criteria of all the identified species is shown above, respectively as Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC) and Data Deficient (DD).

SIGNIFICANCE

This study highlights threatened species found at the Faraday 'Muthi' Market. It also aims to implement viable, educational solutions that will focus on environmental laws and the importance of conserving biodiversity in South Africa.

REFERENCES

- **CBOL Plant Working Groups.** (2009). A DNA barcode of land plants. Proceedings of the National Academy of Sciences USA 106: 12794-12797.
- Doyle, J.J., Doyle, J.L. (1987). A rapid isolation procedure for small amounts of leaf tissue. Phytochemical Bulletin 19,11-14.

Tshisikhawe, M.P. (2002). Trade of indigenous medicinal plants in the Northern Province Venda region.

Williams V.L., Balkwill K., Witkowski E.T.F. (2000). Unraveling the commercial market for medicinal plants and plant parts on the Witwatersrand, South Africa. Economic Botany 54:310–327. doi: 10.1007/BF02864784.