NIR: an invaluable tool for the quality control of Devil's Claw (*Harpagophytum procumbens*)

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Introduction

Harpagophytum procumbens, commonly known as Devil's Claw, is a prostrate, perennial herb, indigenous to Southern Africa.[1] The tuberous secondary roots are purported to possess anti-inflammatory and analgesic activity.[2–4] The roots are typically harvested by hand, sliced and then sun or tunnel-dried and exported without further processing. The dried product can then be ground to a powder which may be infused to produce a herbal tea. The medicinal properties of these roots have been linked to its iridoid glucoside content and in particular to the iridoid, harpagoside (HS).[4,5]

The ratio between HS and another iridoid, 8-ρ-coumaroyl harpagide (8ρCHG) may be used to distinguish *H. procumbens* from a second species, *H. zeyheri*, that is purported not to have medicinal effect.[4,6] This is of importance since Devil's Claw has an established economic value with approximately 600 tons exported per annum.[7,8] According to Chrubasik and Eisenberg[9] suppliers of Devil's Claw are under no obligation to declare the iridoid content of their product. The potential for adulteration exists within this framework and there is a need to provide an accurate and rapid analysis technique that could assist in developing standardisation protocols.

The aim of this study was to test the ability of NIR calibrations to predict the harpagoside and 8-ρ-coumaroyl harpagide contents in dried, ground *Harpagophytum procumbens* root. Additionally, both HS:8ρCHG ratio and moisture content (MC) calibrations were also tested.

Materials and methods

Preparation of samples

Fresh *H. procumbens* secondary roots from cultivated plants were obtained in three batches from Grassroots Natural Products (Plant Improvement Centre, Gouda, South Africa). All fresh roots were kept under ambient conditions until processing. The roots were sliced transversely into 6 mm thick disks and tunnel-dried.

The conditions for tunnel-drying were set at a dry bulb temperature of 60.0°C and relative humidity of 30%, i.e. a wet bulb temperature of 39.72°C. A purpose-built experimental dehydrator was used for drying at a maximum airspeed of 3 $m.s^{-1}$. The drying time was fixed at 24 h.

To avoid any moisture uptake after drying and before grinding, the samples were individually vacuum sealed in aluminium laminated pouches. Grinding of the samples was accomplished using a

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Retsch rotary mill fitted with a 1 mm sieve size. Samples were stored in 30 mL plastic screw cap vials and sealed in 5 L plastic containers with anhydrous $CaCl_2$ as desiccant.

Extraction

A crude methanol extract was prepared by adding 15 mL of a 70% methanol-water mixture to *ca*. 0.15 g of dried, ground sample into 20 mL Pyrex glass culture tubes with teflon-lined screw caps. The tubes were kept in a water bath at 40°C for a total of 60 min, while being manually shaken at 15 min intervals. Samples were decanted into 15 mL graduated polypropylene centrifuge tubes and centrifuged at 4000 r min^{-1} (1788.8 × g) for 12 min. The supernatant was decanted into 24 mL EPA glass vials with teflon-lined screw caps and stored at *ca* 4°C until analysed.

Moisture determination

Moisture content (MC) was determined gravimetrically in duplicate for all samples (n = 150). An average of 5.197 g ± 0.555 g (mean ± SD) of each dried, ground sample was weighed into nickel moisture pans before drying under vacuum (*ca*. 90 kPa) for 16 h at 70°C.

HPLC measurement

HPLC separations were carried out, in duplicate, using a Waters LC Module 1 plus system, equipped with a single wavelength UV-visible detector. The method was based on the HPLC analysis performed by Feistel and Gaedcke,[10] with detection set at 278 nm and 312 nm for HS and 8ρCHG, respectively. A Phenomenex Prodigy 5 ODS-2 column (150 mm × 4.60 mm) was used with a solvent gradient containing water and methanol.

NIR measurements

A Perkin-Elmer FT-NIR Spectrum IdentiCheck instrument was used to generate diffuse reflectance spectra of each sample. Spectra were measured (16 accumulations) across the wavelength range of 1100 nm to 2500 nm with 2 nm intervals and at a resolution of 16 cm^{-1}.

Additionally, spectra of all samples were also generated on a Foss NIRSystems Model 6500 instrument. The same wavelength range was used to allow for comparison between the calibrations based on the spectral information from each instrument.

Calibrations were performed in both the Simca-P 8.1 (Umetrics AB, Sweden) and The Unscrambler® 6.11 (CAMO ASA, Norway) software packages using partial least squares (PLS) regression. The total sample set of 150 samples was used for independantly validated calibrations based on HS and MC reference values. A subset of 40 samples was used for full cross-validated calibrations based on 8ρCHG content and HS:8ρCHG ratios. The spectra of two *H. zeyheri* samples were included in the HS:8ρCHG ratio data set to test the validity of this ratio in distinguishing between the species.

Results and discussion

A summary of the performance of the various calibrations, as pertaining to the two instruments used, is given in Table 1. The MC calibration (Figure 1) performed admirably and the standard error of prediction (*SEP*) compared well with the standard error of laboratory (*SEL*) of 0.14%. The strong linear relationship reflected in the validation scatter plot that was divided into three arbitrary moisture groups, was confirmed by the high correlation coefficient (r) of 0.988.

Table 1. Summary of the performance of NIR models developed for moisture (MC), harpagoside (HS) and 8-ρ-coumaroyl harpagide (8ρCHG) contents in dried Devil's Claw samples.

	MC (%)	HS (%)		8ρCHG (%)	
Instrument	PerkinElmer	PerkinElmer	Foss	PerkinElmer	Foss
Range	2.44–10.43	0.693–2.244	0.693–2.244	0.069–0.290	0.069–0.290
Mean	4.47	1.348	1.348	0.136	0.136
n (validation)	50	50	50	40	40
SEP/*SECV*	0.24	0.236	0.134	0.035	0.028
No. of factors	4	7	11	6	12
Correlation (r)	0.99	0.64	0.90	0.85	0.91
Bias	0.01	–0.048	0.012	0.000	–0.000

The *SEP* of the HS models did not compare very well with the SEL of 0.035% for the HPLC method. For commercial purposes, however, the NIR models are not required to be as sensitive as the reference method. Standardisation of the quality of Devil's Claw may best be served by the introduction of rapid classification methods. It has been suggested that the root be classified according to HS content into four classes: less than 1.2%, 1.2% to 1.6%, 1.6% to 2.5% and greater than 2.5%.

The calibrations involving individual analytes (HS and 8ρCHG) showed better results when using the Foss data. A possible reason for this may be found in the comparison between the spectra generated on the two instruments (Figure 2). The Foss spectrum has a well defined peak at 2190 nm that is lacking in the PerkinElmer spectrum. Absorbances at this wavelength correlate well with the trans-cinnamoyl and ρ-coumaroyl moieties, in HS and 8ρCHG respectively. Additionally, more principle components were used in the Foss calibrations, but care was taken not to overfit the models. Irrespective of these differences, however, the current models should be sufficient for classification purposes based on HS content.

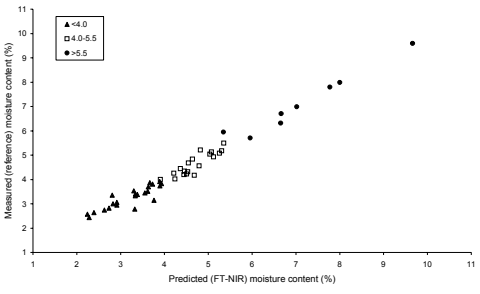

Figure 1. Validation scatter plot (*n* = 50) for the moisture content (MC) of dried, ground Devil's Claw root using FT-NIR data.

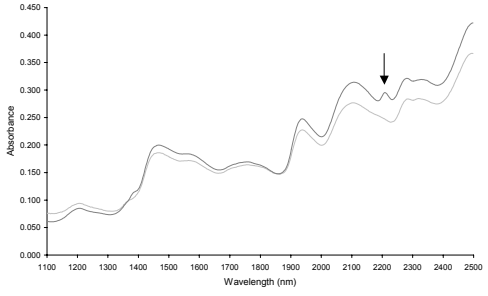

Figure 2. Comparitive NIR spectra of a single Devil's Claw sample scanned on either the PerkinElmer or Foss instruments, with the additional peak at 2190 nm indicated on the Foss spectrum.

Using the HS:8ρCHG ratio (data not shown) as a method to distinguish between *H. procumbens* and *H. zeyheri* samples, this study did not perform as well when compared to similar studies.[4,6] It was found that the ratios of the two *H. zeyheri* samples (both 1.84) were not separated well enough from the overall *H. procumbens* range (5.62 to 34.48; average: 15.83) to validate species distinction based solely on this parameter. A large difference between the 8ρCHG content of the two species was, however, observed in the present study and it was concluded that better separation of the species would most probably be found through direct comparison of only this parameter. In addition, principle component analysis (PCA) of the spectral data showed promise for separation between the species (Figure 3). Futher study with an adequate number of authentic *H. zeyheri* samples is, however, recommended to determine the feasibility of PCA in this regard.

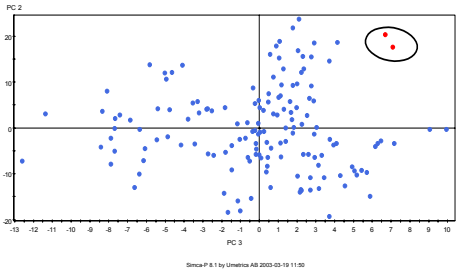

Figure 3. PCA plot of Devil's Claw samples (*n* = 150) indicating possible grouping between *Harpagophytum* species. The two *H. zeyheri* samples are indicated.

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