Rapid screening methods for estimation of mangiferin and xanthone contents of *Cyclopia subternata* plant material

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**Abstract**

Two rapid screening methods, i.e. a colorimetric method employing aluminium chloride complexation and near infrared (NIR) spectroscopy, were evaluated for prediction of the mangiferin and xanthone contents of unfermented *Cyclopia subternata* plant material. Optimum analytical extraction conditions in terms of solvent and time for mangiferin were determined to ensure accurate HPLC reference data. The AlCl3 colorimetric method gave moderate prediction of the mangiferin content (y = 1.3x + 0.87; R² = 0.55). The NIR spectroscopy calibration models developed for prediction of mangiferin (SEP = 0.21 g/100 g; R² = 0.67) and xanthone (SEP = 0.27 g/100 g; R² = 0.66) contents are suitable for screening purposes. To improve the robustness of the NIR spectroscopy calibration the model data set were expanded to include data of unfermented *Cyclopia genistoides*, having higher xanthone content. This did not improve the NIR spectroscopy calibration for prediction of *C. subternata* samples, although the calibration was more robust for prediction of *C. genistoides* samples. Using principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA) it was possible to clearly differentiate between the two species.

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**Keywords:** AlCl3 complexation; Extraction; Hesperidin; Isomangiferin; Mangiferin; NIR spectroscopy; Xanthone

1. Introduction

Standardised traditional herbal formulations remain valid alternatives to single molecule therapy (Patwardhan and Mashelkar, 2009; Schmidt et al., 2007). In many instances the medicinal value of plant extracts could be attributed to their polyphenol content. One compound of interest is mangiferin (Fig. 1), known for various pharmacological effects (Wauthoz et al., 2007); amongst others, immunoprotective (Muruganandan et al., 2005), anti-inflammatory (Leiro et al., 2003), cytoprotective, antigenotoxic (Rao et al., 2009), anti-diabetic and hypolipidemic activities (Dineshkumar et al., 2010).

The endemic South Africa genus *Cyclopia* (family Fabaceae; tribe Podalyrieae), traditionally used as herbal tea, is a good source of mangiferin (Joubert et al., 2008a). Recently, the anti-diabetic potential of *Cyclopia* aqueous extract has been demonstrated (Mose Larsen et al., 2008; Muller et al., 2011). As commercialisation of *Cyclopia* commenced less than two decades ago, cultivation is still limited (Joubert et al., 2011). This, together with increasing demand for the plant material as herbal tea and for extract production, necessitates the use of more than one species in the extract manufacturing process. Considering sustainability, the extract manufacturer is limited to *Cyclopia subternata* and *Cyclopia genistoides*, the only species currently cultivated in substantial quantities. Analysis of aqueous extracts of these two species showed that *C. subternata* contains substantially less mangiferin than *C. genistoides* (De Beer and Joubert, 2010; Joubert et al., 2008b). An investigation of *C. subternata* showed...
that proper drying and storage of the plant material is required to optimise retention of mangiferin (Joubert et al., 2010).

For production of a standardised aqueous extract, containing a specified level of mangiferin, it would be valuable to know the extent of variation of mangiferin content that could be expected in the plant material so that extract specifications could be met. In an industrial processing environment, it is critical to employ analytical methods that would enable rapid prediction of the mangiferin content of the incoming plant material. Quantities of plant material of the different species used for extraction could then be adapted so that the required level of mangiferin in the final extract could be realised.

The applicability of the AlCl3 colorimetric assay for determination of the xanthone content and the use of near infrared (NIR) spectroscopy for estimating the mangiferin content of *C. genistoides* have previously been demonstrated (Joubert et al., 2006, 2008c). Complexation of Al3+ resulted in characteristic bathochromic and hyperchromic shifts in the UV–vis spectrum of mangiferin. When employed for quantification of the mangiferin content of methanol extracts of *C. genistoides*, good correlation (r=0.9) between the HPLC reference data and colorimetric data was observed. The low quantities of mangiferin in *C. subternata* (Joubert et al., 2008b) and other compositional differences between *C. subternata* and *C. genistoides* may pose a challenge in the applicability of these assays for predicting the mangiferin content of *C. subternata*.

The objective of the present study was to evaluate the suitability of both the AlCl3 colorimetric assay and NIR spectroscopy for predicting the mangiferin content of *C. subternata*. The prediction of mangiferin using NIR spectroscopy in a combined data set of both *C. genistoides* and *C. subternata* was also attempted. Since the AlCl3 and the NIR spectroscopy methods are not specific, and interference from other compounds could be a problem, hesperidin was also quantified in all plant material samples. Isomangiferin (Fig. 1), a regio-isomer of mangiferin, and present in a substantially lower concentration in *C. subternata* (De Beer and Joubert, 2010; Joubert et al., 2008b), was included to allow prediction models for xanthone (mangiferin + isomangiferin) content to be established. The total polyphenol content of the plant material was also determined, because it is used in many instances as a quick screening assay of plant material and it is already employed by one of the extract manufacturers as a quality parameter.

With the low mangiferin content of *C. subternata* it was essential that analytical extraction conditions in terms of solvent and time be optimised for mangiferin and especially since the results obtained were to be used as reference data for developing quantitative NIR spectroscopy calibration models. Solvents previously employed for extraction of mangiferin from various plant materials were water, acetone, ethanol and methanol, as well as water–organic solvent mixtures (Baretto et al., 2008; Ling et al., 2009; Núñez-Sellès et al., 2002; Schieber et al., 2000; Zhou et al., 2007). The solvents evaluated in the present study were methanol, ethanol, water and organic solvent–water mixtures, including an acetonitrile–water mixture.

Finally principal component analysis (PCA) and partial least squares (PLS) discriminant analysis were applied to the NIR spectral data to attempt differentiation between *C. subternata* and *C. genistoides* using NIR spectroscopy.

2. Materials and methods

2.1. Chemicals

Solvents for sample preparation and chromatographic separation were of analytical (Analar) and HPLC grades, respectively. Mangiferin, gallic acid, dimethyl sulfoxide (DMSO) (99.5%), acetonitrile R Chromasolv® (Riedel-de Haën) and glacial acetic acid (99.8%) (Riedel-de Haën) were purchased from Sigma-Aldrich (Cape Town, South Africa); sodium carbonate and Folin’s reagent from Merck (Cape Town, South Africa); and ethanol (99%) from Illovo (Cape Town, South Africa). A Modulab Water Purification System (Separations, Cape Town, South Africa) was used to prepare deionised water, which was further purified by means of a Milli-Q 185 Academic Plus water purification system (Microsep (Pty) Ltd., Bellville, South Africa) for HPLC solvent preparation.

2.2. Plant material

Freshly harvested *C. subternata* plant material was dried intact at 40 °C in a temperature-controlled drying tunnel with forced air circulation to ca 8–10% moisture content and ground with a Retsch mill (1 mm sieve; Retsch GmbH, Haan, Germany). A large selection of plant material samples (n=197) was made up from individual cultivated plants harvested during 2004 and 2005 at Kanetberg Flora (Barrydale district, South Africa) and samples obtained from the collection of the Agricultural Research Council (ARC) Infruitec-Nietvoorbij. Samples, consisting of either leaves or stems were also prepared by separating the leaves from the stems after drying, before grinding them separately. These samples were included to extend the range of mangiferin content in the sample set.

2.3. Effect of solvent and extraction time on extraction efficiency

One batch of plant material was randomly chosen for extraction experiments. The ground, dried green plant material (ca 5 g) was weighed in triplicate in 100 mL volumetric flasks to which ca 50 mL of solvent was added. Extractions, performed for 30 min, were carried out on a steam bath when using water and acetonitrile–water (1:2, v/v) as solvents. A water bath (ca 64 °C) was also used for extraction with acetonitrile–water (1:2, v/v) and the other solvents, i.e. ethanol–water (1:1, v/v; 4:1, v/v), methanol–water (1:1, v/v), ethanol and methanol. After cooling to room temperature either water or the respective organic solvent was used to fill the respective volumetric flasks to volume. The extracts were filtered using Whatman no. 4 filter paper (Whatman International Ltd., Maidstone, UK). The experiment was repeated as blocks on three separate days.

The effect of time (10, 20, 30 and 40 min) on extraction efficiency was determined using 5 g plant material, the acetonitrile–water (1:2) mixture and heating on a steam bath.
Three replicate extractions were carried out for each time period. Aliquots of all extracts were stored at −18 °C until analysed.

2.4. Extract preparation for calibration model development

The samples (n=197) used for development of the AlCl₃ calibration curve and NIR spectroscopy calibration models were extracted for 20 min on a steam bath with the acetonitrile-water mixture as described earlier (Section 2.3). Aliquots were stored at −18 °C until analysed.

2.5. Quantification of mangiferin, isomangiferin and hesperidin contents by HPLC analysis

The extract was filtered directly prior to HPLC analysis using a 0.45 μm Millipore Millex-HV hydrophilic PVDF syringe filter (25 mm diameter) (Microsep (Pty) Ltd). Quantification of mangiferin in the extracts (10 μL) was performed in duplicate by reversed-phase HPLC. The equipment consisted of a LaChrom (Merck/Hitachi) HPLC (L7000 interface, L7400 UV detector, L7450 diode-array detector (DAD), L7100 pump and L7200 autosampler) (Merck, Cape Town, South Africa), in-line vacuum degasser (Model DG-4400, Phenomenex, Santa Clara, California) and Varian HPLC 5000 column oven (Varian, Inc., Palo Alto, USA) controlled by a CAL 3200 temperature controller (CAL Controls Ltd, Herts, UK). Separation was performed at 30 °C on a Phenomenex Luna Phenyl Hexyl column (3 μm, pore size 100 Å, 150×4.6 mm) using binary gradient separation with acetic acid–water (2:98, v/v) and acetonitrile at a flow rate of 1 mL/min (Program I of Joubert et al., 2003). The DAD was used to check peak purity. The absorbance measurements at 320 nm (xanthones) and 288 nm (hesperidin), performed with the UV detector, were used for quantification. Standard dilution series of mangiferin (concentration range 0.15–10.9 μg injected; R²=1) and hesperidin (0.1–6 μg injected; R²=1) were prepared and analysed weekly. A typical HPLC chromatogram of a C. subternata extract is shown in Fig. 2. Isomangiferin was quantified in terms of mangiferin equivalents. Integration of the peak area was done with the LaChrom Multisystem Software D700. Results were expressed on a dry plant material basis.

2.6. Colorimetric determination of xanthone and total polyphenol contents

The xanthone and total polyphenol contents, using AlCl₃ and the Folin–Ciocalteu reagents, respectively, were determined colorimetrically as described by Joubert et al. (2008c). For quantification of the xanthone content a calibration curve was prepared with mangiferin. The total polyphenol content was quantified as gallic acid equivalents. Results were expressed on a dry plant material basis.

2.7. Near infrared spectroscopy measurements

NIR spectroscopy measurements were performed in diffuse reflectance mode using a Büchi NIRLab N-200 Fourier transform near infrared (FT-NIR) spectrophotometer with NIRLabWare (version 3.0) measurement software (Büchi Labortechnik AG, Flawil, Switzerland). The ground, dried plant material was presented to the instrument in rotating glass Petri dishes and NIR spectra were collected from 1000 to 2500 nm (10,000–4000 cm⁻¹) at a resolution of 8 cm⁻¹ resulting in 1557 data points as a data point was collected every 3.86 cm⁻¹.

2.8. Near infrared spectroscopy calibration development

The Unscrambler® (version 9.2) software (Camo Process AS, Oslo, Norway) was used to develop PLS regression models for the estimation of mangiferin and xanthone contents of C. subternata plant material. C. subternata spectral data (n=132) were pretreated with the multiplicative scatter correction (MSC) algorithm (Geladi et al., 1985) to remove the effects of scattering and to compare the mean spectra from approximately the same baseline, followed by

![Fig. 1. Structures of mangiferin, isomangiferin and hesperidin.](image)

![Fig. 2. Typical HPLC chromatogram of an acetonitrile–water extract of green C. subternata with detection at 320 nm (0–11.6 min) for mangiferin (1) and isomangiferin (2) and 288 nm (11.6–30 min) for hesperidin (3).](image)
Savitsky–Golay first derivative (23 point segment; 2nd order polynomial) (Savitzky and Golay, 1964). Savitzky–Golay derivatives were used to enhance the differences between spectra.

The calibration models were validated by means of independent validation (n=65). A PLS calibration model was also developed on a combined spectral data set (pretreated with MSC) comprising the data of both C. subternata as well as C. genistoides (n=292) with subsequent independent validation (n=145). The C. genistoides spectral data recorded as well as the HPLC reference data as described by Joubert et al. (2006) were used. Extraction of 0.5 g C. genistoides plant material was conducted with 50 mL methanol for 30 min in a water bath at 64 °C. Both validation sets were randomly selected. The standard error of prediction (SEP), coefficient of determination (R²) and RPD (Williams, 2001), that is the Ratio of the standard error of Prediction to the standard Deviation of the validation set (to indicate the efficiency of a calibration), were used to evaluate the accuracy of the calibration models. The aim of model development is to obtain a calibration model with a low SEP, a high R² (above 0.91) and a RPD value higher than 5. The value of SEP should also be as close as possible to the standard error of laboratory (SEL).

2.9. Classification using principal component analysis and PLS discriminant analysis

Principal component analysis (Unscrambler® version 9.2 software) and PLS discriminant analysis were applied to the combined spectral data set of C. genistoides (n=240) and C. subternata (n=197) after MSC and Savitsky–Golay 2nd derivative (15 point segment; 3rd order polynomial) pretreatment of the spectra. For the PLS discriminant analysis the coding 0/1 with a threshold of 0.5 was used. An independent validation set, comprising one third of the data set, was randomly selected.

2.10. Statistical analysis

One-way analysis of variance (ANOVA) was performed with SAS version 9.13. The Student’s t-LSD (Least Significant Differences) was calculated at a 5% level (P<0.05) to compare treatment means. Correlations were undertaken with the parametric Pearson r value and the nonparametric Spearman correlation. Graphs were compiled using STATISTICA version 7.1 (StatSoft, Inc., Tulsa, OK, USA).

3. Results and discussion

3.1. Effect of solvent composition and extraction time on extraction efficiency

The extraction solvent significantly (P<0.5) affected the recovery of total polyphenols, xanthones (Fig. 3), mangiferin and hesperidin (Fig. 4). Total polyphenols were best recovered with acetonitrile–water (1:2; steam bath and water bath) and ethanol–water (1:1). The highest recovery of xanthones was with ethanol–water (1:1) and acetonitrile–water (1:2; water bath). Extraction with acetonitrile–water (1:2) on the water bath did not significantly (P≥0.05) improve extraction of the xanthones relative to extraction performed on the steam bath.

Acetonitrile–water (1:2; steam bath and water bath), methanol–water (1:1) and ethanol–water (1:1) were equally effective in extracting mangiferin. Acetonitrile–water (1:2; steam bath) was also found to give the best extraction of hesperidin from the dried plant material (P<0.05) (Fig. 4). When quantification of both mangiferin and hesperidin contents of C. subternata is important, such as for NIR calibration development, acetonitrile–water (1:2) extraction on a steam bath would thus be the procedure of choice. If the analyst is interested in extracting mangiferin rather than hesperidin, ethanol–water could be used as an alternative solvent (1:1) due to its efficiency in extracting mangiferin. An additional advantage of ethanol–water (1:1), as an extraction solvent, is that it is safe to work with and less expensive than acetonitrile.

Different extraction times showed little effect on extraction of total polyphenols, mangiferin and hesperidin when using acetonitrile–water (1:2; steam bath) (Table 1). Subsequent extractions for NIR spectroscopy calibration model development were standardised on 20 min extraction time to ensure efficient extraction, but prevent unnecessary exposure to heat that could lead to degradation of compounds.

![Graph](image-url)
3.2. AlCl₃ colorimetric method

A moderate correlation ($R^2 = 0.55$) was obtained for the dried, green *C. subternata* samples between the xanthone content determined using the AlCl₃ colorimetric assay and mangiferin content quantified by HPLC (Fig. 5a). Values estimated by AlCl₃ (0.22 to 3.69 g/100 g) were higher than those quantified by HPLC (0.02 to 2.7 g/100 g). The correlation was similar to that obtained for *C. genistoides* ($R^2 = 0.59$) (Joubert et al., 2008b). By including isomangiferin, present in quantities of 0.01 to 0.06 g/100 g to give the xanthone content, the correlation was only slightly improved for *C. subternata* ($R^2 = 0.56$), but the slope decreased substantially (Fig. 5b), showing a more direct relation between the colorimetric and HPLC data. For both mangiferin and xanthone contents the data were mostly clustered over a narrow range. It is not expected that hesperidin would substantially contribute to the overestimation in the case of *C. subternata*, since it was present in low quantities (0.05 to 0.69 g/100 g) and being a flavanone it is not very reactive towards AlCl₃ (Joubert et al., 2008c). The presence of flavones such as scolymoside, meeting the requirements for complexation of Al³⁺ however, will contribute to absorbance (De Beer and Joubert, 2010; Kamara et al., 2004; Kokotkiewicz et al., 2012).

### 3.3. NIR spectroscopy calibration models

A summary of the reference data for mangiferin and xanthone contents of *C. subternata* is given in Table 2. The distribution of mangiferin and xanthone contents is depicted in Fig. 6. A skew distribution to the lower values was noticed for both parameters. If, however, a Gaussian distribution had been obtained, more accurate predictions near the mean and less accurate predictions

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**Table 1**

| Time (min) | Total polyphenol a | Mangiferin a | Hesperidin a |
|------------|-------------------|--------------|--------------|
| 10         | 11.39±0.59        | 1.35±0.06    | 0.58±0.04    |
| 20         | 12.35±0.47        | 1.36±0.04    | 0.61±0.04    |
| 30         | 12.53±0.46        | 1.31±0.17    | 0.58±0.6     |
| 40         | 12.25±0.91        | 1.31±0.15    | 0.59±0.6     |

* Mean (n=3)±SD. Results expressed as g/100 g.
for samples near the extremes of the ranges could be likely (Williams, 2001).

The validation results of the mangiferin calibration model (SEP = 0.21 g/100 g; R² = 0.67; RPD = 1.62) indicated that it can be used for screening and approximate predictions (Table 3; Fig. 7a). The calibration model for estimation of xanthone content (SEP = 0.27 g/100 g; R² = 0.66; RPD = 1.52) gave similar results (Table 3; Fig. 7b). The values compared relatively well with their SEL values obtained (0.03 g/100 g) for mangiferin and (0.07 g/100 g) for xanthones (Table 3). It is, however, to be expected that the accuracy would not be as good as that of the HPLC analysis. A small bias was observed for both mangiferin (0.007%) and xanthone (0.001%) content estimation (Table 3). The similarity in results obtained for the prediction of mangiferin and xanthone contents might be due to the solubility of these compounds. Mangiferin and isomangiferin only differ in the position of the glucose moiety on the 9H-xanthen-9-one (dibenzo-γ-pirone) nucleus. Isomangiferin could have slightly affected the accuracy of the calibration.

The calibration model for the mangiferin content of *C. subternata* was weaker in comparison to that obtained by Joubert et al. (2006) for *C. genistoides* (Table 3). The latter study used a slightly larger sample set (n = 240) compared to that of the present study (n = 197). Although smaller SEP values were obtained for the *C. subternata* models compared to the *C. genistoides* models (Joubert et al., 2006), the larger RPD values obtained in the latter study suggested a more robust calibration model for *C. genistoides*. The average mangiferin content for *C. subternata* was substantially lower than for *C. genistoides*. The smaller range in mangiferin content (0.06 to 3.11 g/100 g) of the *C. subternata* sample set compared to that (0.70 to 7.21 g/100 g) of the *C. genistoides* sample set (Joubert et al., 2006) contributed to a less robust calibration and subsequently the prediction ability of the model was impeded.

Combining the two spectral data sets of *C. subternata* and *C. genistoides* to develop a calibration model for the estimation of mangiferin resulted in a more robust calibration with a RPD of 3.92 indicating that the calibration would be suitable for quality control purposes (Tables 2 and 3; Fig. 8). This calibration would particularly be suitable for future *C. genistoides* samples. The decrease in accuracy (higher SEP) in comparison to the model for prediction of mangiferin content of only *C. subternata* samples indicates that the calibration model developed from the combined data set would probably not be suitable for *C. subternata* samples in a quality control environment.

Table 2

Reference data of ground, dried green honeybush plant material for mangiferin and xanthone contents of *C. subternata*, mangiferin content of *C. genistoides* and mangiferin content of a combined data set of *C. subternata* and *C. genistoides*.

|                      | C. subternata | C. genistoides | C. subternata and C. genistoides |
|----------------------|--------------|----------------|---------------------------------|
|                      | Mangiferin   | Xanthon        | Mangiferin                      | Mangiferin |
| Calibration set      |              |                |                                 |
| Range (g/100 g)      | 0.06–3.11    | 0.08–3.66      | 0.70–7.21                       | 0.06–7.21  |
| Mean (g/100 g)       | 0.57         | 0.73           | 3.64                            | 2.36       |
| SD                   | 0.40         | 0.49           | –                               | 1.68       |
| n                    | 132          | 132            | 160                             | 292        |
| Validation set       |              |                |                                 |
| Range (g/100 g)      | 0.07–2.19    | 0.11–2.60      | 1.17–7.18                       | 0.11–5.64  |
| Mean (g/100 g)       | 0.51         | 0.65           | 3.68                            | 2.25       |
| SD                   | 0.34         | 0.41           | 0.90                            | 1.57       |
| n                    | 65           | 65             | 80                              | 145        |
| SEL b                | 0.03         | 0.07           | 0.08                            | –          |

a Joubert et al. (2006).

b Standard error of laboratory for reference data of complete data set.

Fig. 6. Histogram of the distribution of (a) mangiferin and (b) xanthone contents of the ground, dried green *C. subternata* plant material samples (calibration and validation sets combined).
C. genistoides extracts have been prepared with methanol which might have resulted in the mangiferin content of the reference data being slightly underestimated compared to that of C. subternata. This could have affected the accuracy of the combined calibration to some extent.

3.4. Classification using principal component analysis and PLS discriminant analysis

Typical raw (no pretreatment) NIR spectra of ground, dried green C. subternata, as well as C. genistoides plant material are shown in Fig. 9a. After application of MSC (Fig. 9b), differences could already be seen in the spectral data. Distinct differences between the two species were evident in the spectral regions from 2050 to 2150 nm and from 2250 to 2350 nm.

The PCA score plots (PC1 vs PC2) of the spectral data of C. subternata and C. genistoides samples pretreated with MSC (majority of particle size differences removed) showed two clear clusters, although with a slight overlap between some of the samples (Fig. 10a). Better clustering was, however, observed when the spectral data were pretreated with MSC followed by 2nd derivative (15 point segment; 3rd order polynomial) (Fig. 10b).

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Table 3
NIR spectroscopy validation results for the prediction of mangiferin and xanthone contents in ground, dried green honeybush plant material for C. subternata, mangiferin content for C. genistoides and mangiferin content for a combined data set of C. subternata and C. genistoides.

|                        | C. subternata | C. genistoides | C. subternata and C. genistoides |
|------------------------|--------------|----------------|----------------------------------|
| **Mangiferin**         |              |                |                                  |
| SEP (g/100 g)          | 0.21         | 0.46           | 0.21                             |
| R²                     | 0.67         | 0.74           | 0.67                             |
| Bias                   | 0.007        | -0.04          | 0.007                            |
| PLS factors            | 8            | 4              | 8                                |
| RPD                    | 1.62         | 1.96           | 1.62                             |
| **Xanthone**           |              |                |                                  |
| SEP (g/100 g)          | 0.27         | 0.40           | 0.27                             |
| R²                     | 0.66         | 0.94           | 0.66                             |
| Bias                   | 0.001        | 0.003          | 0.001                            |
| PLS factors            | 8            | 12             | 8                                |
| RPD                    | 1.52         | 3.92           | 1.52                             |

a Joubert et al. (2006).
b Standard error of prediction.
c Number of PLS factors.
d Ratio of standard error of prediction to standard deviation of validation set.
Pretreatment was considered (and shown to be) necessary as the different grinding properties of two species, with *C. subternata* having a higher woody content, could have contributed to clustering. MSC was applied (Geladi et al., 1985), which is based on the fact that physical light scatter has wavelength dependencies different from that of chemically absorbed light and thus allows separation of chemically absorbed light from physical light scatter. Using many wavelengths, it is possible to distinguish between chemical absorption and scatter. During the MSC calculation, the scatter for each sample is estimated relative to that of an ideal sample. The spectrum of each sample is then corrected so that all samples appear to have the same scatter level as the ideal.

Derivatives, on the other hand, are used to separate overlapping peaks. Applying derivatives led to an increase in spectral resolution, with a subsequent increase in the signal-to-noise ratio. An increase in noise was avoided by smoothing the spectra using the Savitzky–Golay algorithm (Savitzky and Golay, 1964), which is a moving window averaging method. A window is selected where the data are fitted by a polynomial; the central point in the window is replaced by the value calculated from the polynomial. The use of pretreatment methods thus ensured that classification was due to chemical differences between the species, including quantitative and qualitative differences.

PCA loading line plots are usually used to explain the variation on which the classification between samples is based.

For ease of interpretation the loading line plots of PC1 and PC2 of the MSC pretreated data have been used in this case (Fig. 11). Both PC1 and PC2 seem to be necessary to classify between the two *Cyclopia* spp. With the classification mostly in the direction of PC2, the spectral region between 2250 and 2350 nm is shown to be contributing. The loading values of these absorption bands, as well as the PC score values, are negative for *C. genistoides*. Wavelengths within this range were also shown to be the major absorption bands in *C. genistoides* in an earlier study (Joubert et al., 2006), i.e. 2000 nm (2×O–H deformation and C–O deformation), 2200 nm (C–H stretch and C==O stretch), 2280 nm (C–H stretch and C–H deformation) and 2360 nm (O–H deformation second overtone). A strong absorption at ca 1940 nm (O–H stretch and O–H deformation) was also present. This wavelength region corresponds most strongly to water; in this study the moisture content of the samples was controlled to be...
similar and constant. Thus difference in moisture would not have contributed to the classification.

Developing PLS discriminant analysis models from the pretreated data, 100% correct classification was observed in the validation set.

4. Conclusions

Correlations observed between the mangiferin and xanthone contents quantified by HPLC and AlCl₃ colorimetric method were as expected and similar to results observed in previous studies of C. genistoides. When sophisticated laboratory equipment is not available, the colorimetric method could be used for screening of the plant material.

NIR spectroscopy calibration models for estimation of mangiferin and xanthone contents of C. subternata were also shown to be suitable only for screening purposes. Although the isomangiferin content is present in much smaller quantities than mangiferin, it contributed towards the xanthone content and accuracy of the calibration models. Improvement of the calibration model could be achieved by adding more samples with higher mangiferin content to give a more even distribution of the mangiferin and xanthone contents. Developing calibration models with a combined data set of both species resulted in a more robust calibration for mangiferin estimation in C. genistoides, but not in C. subternata.

Using NIR spectroscopy it is possible to distinguish between ground, dried plant material of green C. subternata and green C. genistoides either based on particle size and/or chemical differences.

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