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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 $AlCl_3$ colorimetric method gave moderate prediction of the mangiferin content ($y=1.3x+0.87$; $R^2=0.55$). The NIR spectroscopy calibration models developed for prediction of mangiferin (SEP=0.21 g/100 g; $R^2=0.67$) and xanthone (SEP=0.27 g/100 g; $R^2=0.66$) 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.

Keywords: $AlCl_3$ 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

Abbreviations: FT-NIR, Fourier transform near infrared; MSC, Multiplicative scatter correction; NIR, Near infrared; PCA, Principal component analysis; PLS, Partial least square; R^2, Coefficient of determination; SD, Standard deviation; SEL, Standard error of laboratory; SEP, Standard error of prediction.

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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 $AlCl_3$ 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 Al^{3+} 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 $AlCl_3$ 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 $AlCl_3$ 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-Sellés 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 Académic 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.