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Original Article

Distribution patterns of flavonoids from three *Momordica* species by ultra-high performance liquid chromatography quadrupole time of flight mass spectrometry: a metabolomic profiling approach

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ABSTRACT

Plants from the *Momordica* genus, Curcubitaceae, are used for several purposes, especially for their nutritional and medicinal properties. Commonly known as bitter gourds, melon and cucumber, these plants are characterized by a bitter taste owing to the large content of cucurbitacin compounds. However, several reports have shown an undisputed correlation between the therapeutic activities and polyphenolic flavonoid content. Using ultra-high performance liquid chromatography quadrupole time of flight mass spectrometry in combination with multivariate data models such as principal component analysis and hierarchical cluster analysis, three *Momordica* species (*M. foetida* Schumach., *M. charantia* L. and *M. balsamina* L.) were chemo-taxonomically grouped based on their flavonoid content. Using a conventional mass spectrometric-based approach, thirteen flavonoids were tentatively identified and the three species were found to contain different isomers of the quercetin-, kaempferol- and isorhamnetin-*O*-glycosides. Our results indicate that *Momordica* species are overall very rich sources of flavonoids but do contain different forms thereof. Furthermore, to the best of our knowledge, this is a first report on the flavonoid content of *M. balsamina* L.

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Introduction

Momordica species are versatile plants belonging to the family Cucurbitaceae and are often referred to by various common names such as bitter gourd, bitter cucumber and bitter melon (Nagarani et al., 2014a). In different parts of the world, *Momordica* plants are consumed as a vegetable and are known for their bitter taste due to the presence of phytochemicals such as alkaloids and cucurbitacins (Chen et al., 2005; Rios et al., 2005; Nagarani et al., 2014a). Within this genus there are several species widely distributed across the globe, mainly in the tropical and subtropical regions of Africa, Asia and Australia. In a recent review article by Nagarani et al. (2014a), undisputed scientific evidence on the origin of these plants was presented and believed to be endemic to India, while earlier biogeographical origins of these species can be found elsewhere (Dey et al., 2006; Singh et al., 2007; Gaikwad et al., 2008).

Apart from the nutritious value, *Momordica* species are also used for their medicinal properties. For example, *M. charantia* has been used in Chinese folk medicine for the treatment of different chronic diseases (Zhang, 1992). In some parts of South Africa, these species are currently used as medication for sugar diabetes and chronic hypertension diseases, without any scientific backing. Due to the wide range of phytochemicals in *Momordica* (Nagarani et al., 2014a), it is very difficult to point out one active compound, even though numerous metabolites with known pharmacological activities have been identified (Singh et al., 2011; Kenny et al., 2013; Nagarani et al., 2014b). *Momordica* species are known to contain large quantities of polyphenolic compounds (Kubola and Siriamornpun, 2008) and amongst these are flavonoids which are known to possess several therapeutic activities. Further reports have suggested a possible link between the medicinal properties of *Momordica* species and their flavonoid content (Horax et al., 2005; Lin and Tang, 2007; Wu and Ng, 2008; Zhu et al., 2012). The distribution of flavonoids in these plants represents another interesting dimension. For instance, Kenny et al. (2013) reported the presence of flavonoids in the bitter melon (*M. charantia*) fruit, however, the levels vary across different sections of the fruit. In a separate study, Nagarani et al. (2014b) reported a very interesting distribution pattern across different species of *Momordica* (*M. tuberosa*, *M. charantia* and *M. cochinchinensis*). Here, the flavonoid rutin was detected in

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the former two species but absent in the later. As such, the aim of the current study was to investigate the flavonoid distribution patterns within the three *Momordica* species (namely *M. charantia* L., *M. foetida* Schumach. and *M. balsamina* L.) currently used for diabetic control and nutritional value in the Limpopo (Northern) regions of South Africa.

In addition to their nutritional and medicinal properties, plant metabolites can also be used to taxonomically classify plants to ensure that the correct specie(s) are used for medicinal purposes. In the past, flavonoids have been used for chemo-taxonomical classification of plants (Iwashina, 2000; El Shabrawy et al., 2014; Martucci et al., 2014). To achieve our objective, the current study was divided into two parts: the first aiming to establish a chemo-taxonomical relationship between the three species using a metabolomic profiling approach with the aid of UHPLC-qTOF-MS and multivariate data models, and in the second, the flavonoid composition of the three species was investigated using targeted MS-based flavonoid identification strategies presented elsewhere (Cuyckens and Claeys, 2004).

Materials and methods

Plant materials and chemicals

Momordica plants, Curcubitaceae, were collected in and around the Venda region of South Africa with the help of the local farmers. Briefly, *M. charantia* L. was collected from a farm in the Nwanedi farming area, about 80 km south of the Zimbabwean border. The other two species, *M. balsamina* L. and *M. foetida* Schumach. were collected from various villages around Thohoyandou. The species were identified with the help of Mr Philip Ramela (Madzivhandila College of Agriculture, South Africa) and for further confirmation, the plant materials were also compared to the national herbarium specimens at the South African National Biodiversity Institute (SANBI) (Pretoria, South Africa). Voucher herbarium specimens (with voucher number NEM003 (*M. balsamina*), NEM004 (*M. charantia*) and NEM005 (*M. foetida*)) were prepared and deposited to the Department of Botany, University of Johannesburg. Unless stated otherwise, all the chemicals were of analytical grade and obtained from various internationally reputable suppliers. Both the methanol and acetonitrile (Romil, MicroSep, South Africa) were used for UHPLC-qTOF-MS analyses. Water was purified with a Milli-Q Gradient A10 system (Millipore, Billerica, MA, USA). Leucine encephalin, rutin and formic acid were purchased from Sigma Aldrich, Germany. Quercetin-3-glucose, quercetin-4′-glucose and quercetin-7-glucose were purchased from Phytolab (Vestenbergsgreuth, Germany).

Metabolite extraction

The leaves of the three *Momordica* species were air-dried at 37 °C for three consecutive days. Metabolites were extracted from the four independently crushed leaf samples (2 g), representing four independent biological replicates, using 80% aqueous methanol (20 ml). For maximum extraction, the homogenate was placed on an orbital shaker at room temperature (25 °C) for at least 30 min. After the extraction, the tissue debris was removed by centrifugation at $5000 \times g$ for 10 min. The supernatant was dried to at least 1 ml using a rotary evaporator operating at 55 °C under negative pressure vacuum. The 1 ml extract was subsequently dried to completeness using a vacuum concentrator centrifuge (Vacufuge, Eppendorf, Germany) operating at 55 °C. Prior to UHPLC–MS analyses, the pelleted extract residues were re-constituted in 1 ml of 50% aqueous methanol and filtered through 0.22 μm nylon filters.

UHPLC–MS analyses

For UHPLC–MS analyses, a previously described method (Madala et al., 2014a) was used. Briefly, one (1) μl of the extracts was analysed on a Waters Acquity BEH C8 column (150 mm × 2.1 mm, 1.7 μm particle size) and the temperature controlled at 60 °C. Here, three technical replicates were analyzed and randomized during the UHPLC–MS analyses using online randomizing software (www.random.org/lists/) to avoid technical bias. A binary solvent mixture was used consisting of water containing 10 mM formic acid (pH 2.3) (eluent A) and acetonitrile containing 10 mM formic acid (eluent B). The initial conditions were 98% A at a flow rate of 0.4 ml/min and maintained for 1 min, followed by multiple gradients to 5% A at 26 min. The conditions were kept constant for 1 min and then changed to the initial conditions. The total chromatographic run time was 30 min. Chromatographic elution was monitored with the aid of a photo diode array (PDA) detector and MS.

For MS detection, a high resolution mass spectrometer (Waters SYNAPT G1 Q-TOF system), operating in V-optics and electrospray negative mode, was used. Leucine enkephalin (50 pg/ml) was used as reference lockmass calibrant to obtain typical mass accuracies between 1 and 5 mDa. The optimal conditions for analysis were as follows: capillary voltage of 2.5 kV, the sampling cone at 30 V and the extraction cone at 4 V. The scan time was 0.1 s covering the 100–1000 Da mass range. The source temperature was 120 °C and the desolvation temperature was set at 450 °C. Nitrogen was used as the nebulization gas at a flow rate of 700 l/h. To obtain better metabolite coverage and fragmentation patterns thereof, the MS was operated at different collision energy (CE) levels as reported elsewhere (Madala et al., 2012). For comparison purposes, authentic standards (quercetin-3-glucose, quercetin-4′-glucose and quercetin-7-glucose) were also analyzed using the same conditions. All the acquisition and analysis of data were controlled by Waters MassLynx^{TM} v4.1 software (SCN 704).

Multivariate data analyses

Primary raw data was analyzed by data alignment, peak finding, peak integration and retention time (Rt) correction using a Markerlynx XS^{TM} software (Waters Corporation, Milford, USA) with the following processing parameters: Rt range of 7–12 min, mass range of 100–1000 Da, mass tolerance of 0.02 Da, Rt window of 0.2 min. The resulting datasets were exported to the SIMCA-P software version 13.0 (Umetrics, Umea, Sweden) for Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA). Before the models were computed, all the data were mean centered and *Pareto*-scaled. For HCA analysis, the Ward distance algorithm was used to calculate the distance between the different generated clusters (Madala et al., 2014b).

Results and discussion

Classification of Momordica *species based on their flavonoid content*

Analyses of the crude aqueous-methanol extracts prepared from the leaves of the three *Momordica* species were conducted using an UHPLC-qTOF-MS operating in negative electrospray ionization (ESI) mode. The data obtained was automatically processed by MarkerLnx^{TM} software targeting the flavonoid region (7–12 min) of the chromatograms (Fig. 1). The resulting files were further exported to the SIMCA-P version 13 software for multivariate data analyses. The resulting PCA and HCA are shown in Fig. 2. In the past it was noted that there seems to exist a tendency amongst

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