South African Journal of Botany 108 (2017) 193–199

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

UPLC-qTOF-MS profiling of pharmacologically important chlorogenic acids and associated glycosides in *Moringa ovalifolia* leaf extracts

C. Makita [a], L. Chimuka [a], E. Cukrowska [a], P.A. Steenkamp [b,c], M. Kandawa-Schutz [d], A.R. Ndhlala [e], N.E. Madala [b,*]

[a] Molecular Sciences Institute, School of Chemistry, University of the Witwatersrand, P/B 3, 2050, Johannesburg, South Africa
[b] Department of Biochemistry, University of Johannesburg, P.O. Box 524, Auckland Park, 2006 Johannesburg, South Africa
[c] CSIR Biosciences, Natural Products and Agroprocessing Group, Pretoria 0001, South Africa
[d] School of Chemistry, University of Namibia, P/B 13301 Windhoek, Namibia
[e] Agricultural Research Council, Vegetable and Ornamental Plants (VOP), Private Bag X293, Pretoria 0001, South Africa

ARTICLE INFO

Article history:
Received 13 April 2016
Received in revised form 30 August 2016
Accepted 9 October 2016
Available online xxxx

Edited by K Doležal

Keywords:
Moringa ovalifolia
UPLC-qTOF-MS
Chlorogenic acids
Caffeoylquinic acids
Dicaffeoylquinic acids
p-Coumaroylquinic acids
Feruloylquinic acids
Caffeoylquinic acid glycosides

ABSTRACT

Moringa ovalifolia Dinter & A. Berger (Moringaceae) is a succulent-stemmed plant, endemic to the desert and semi-desert areas of central Namibia and southwestern Angola. Just like other species in the Moringaceae family, *M. ovalifolia* is believed to be rich in health-promoting phytochemicals. However, there are very limited scientific reports on the phytochemical composition and associated biological activities of this plant. Chlorogenic acids (CGAs), major phenolic compounds of *Moringa* species, have been shown to be effective natural remedies for the management of chronic ailments such as diabetes and cardiovascular diseases. Using a UPLC-ISCID-MS/MS method optimized to mimic the MS^n fragmentation of an ion trap-based MS but generating accurate mass data, various isomers of chlorogenic acids and their associated derivatives in the leaves of *M. ovalifolia* were profiled. *M. ovalifolia* was shown to contain *cis* and *trans* isomers of 3-acyl, 4-acyl and 5-acyl *p*-coumaroylquinic (*p*CoQA), caffeoylquinic (CQA) and feruloylquinic acids (FQA) (**1**–**18**), a single isomer of 3,5-*di*CQA (**19**), 3-CQA-glycoside (**20**) and two regional isomers of the (3′ and 4′) glycosides of 4-CQA (**21, 22**). To the best of our knowledge, this is the first report on the presence of these compounds in *M. ovalifolia*. The results of the current study confirmed the richness of an underutilized *M. ovalifolia* as a source of pharmacological relevant metabolites with potential medicinal applications.

1. Introduction

Chlorogenic acids (CGAs) are natural antioxidants and form part of the most abundant polyphenols in the human diet. They are usually produced by plants as a part of the defence mechanism response against environmental stresses triggered by microbial pathogens, mechanical wounding and direct exposure to UV or visible light (Clifford, 1999, 2000; Madala et al., 2014). CGA are esters of one or more cinnamic acids and quinic acid. The most common cinnamic acids encountered are caffeic, *p*-coumaric and ferulic acid, which give rise to *p*-coumaroylquinic (*p*CoQA), caffeoylquinic (CQA) and feruloylquinic acids (FQA) (Clifford, 1999, 2000). The *trans*-isomers are predominant but *cis*-isomers are known particularly in tissue subject to intense UV irradiation (Clifford et al., 2008). Apart from being strong antioxidants, CGAs and their derivatives are of particular interest as several epidemiological studies have associated a diet rich therein with certain health-promoting properties such as anti-inflammatory, anti-diabetes, anti-viral and anti-cancer activities (Eliel and Ramirez, 1997).

Moringa ovalifolia is a succulent-stemmed plant belonging to the family Moringaceae. It is endemic to the dry, desert and semi-desert areas of central Namibia and southwestern Angola where the species is utilized for several medical and malnutritional conditions. Just like the other species in the family, *M. ovalifolia* is rich in protein, calcium, iron and vitamin C. In many parts of the world, several *Moringa* species are consumed as a nutritional supplement in the form of capsules or leaf powder (Pakade et al., 2013; Khoza et al., 2016). The family Moringaceae is known for its exceptional nutritional values and healing purposes (Pakade et al., 2013). Information on pharmacological activities, phytochemical composition and nutritional values of *M. ovalifolia* is scarce. Most researchers concentrate on the most famous species within the family, *Moringa oleifera* (Makita et al., 2016). Analytical studies have identified a range of phytochemicals, including glucosinolates, sterols, proanthocyanidins, flavonoids and cinnamates in various tissues and organs (Bennett et al., 2003; Ncube et al., 2014; Ramabulana et al., 2015; Khoza et al., 2016; Makita et al., 2016; Mhlongo et al., 2016). It is assumed but not proven that these

* Corresponding author.
E-mail address: emadala@uj.ac.za (N.E. Madala).

http://dx.doi.org/10.1016/j.sajb.2016.10.016
0254-6299/

phytochemicals are also responsible for the perceived benefits posed by members of the *Moringa* species (Siddhuraju and Becker, 2003; Ndong et al., 2007; Verma et al., 2009; Kashiwada et al., 2012; Vongsak et al., 2013a, 2013b, 2013c).

This study was aimed at profiling pharmacologically important chlorogenic acids and associated glycosides (cinnamates) in *M. ovalifolia* with the aid of a recently developed in-source collision-induced dissociation approach of ultra-performance liquid chromatography mass spectrometry technique.

2. Materials and methods

2.1. Chemicals

All chemicals utilized in this study were of analytical grade quality and were obtained from various international suppliers. Briefly, the organic solvents utilized were UPLC/MS grade quality methanol (Romil, MicroSep, South Africa) and acetonitrile (Romil, MicroSep, South Africa). Water was purified by a Milli-Q Gradient A10 system (Millipore, Billerica, MA, USA). Leucine encephalin and formic acid were all purchased from Sigma Aldrich, Germany. The alcoholic cider (Savanna) and coffee were purchased from local liquior store and food outlet, respectively. The caffeoylquinic standards were purchased from PhytoLab, Germany.

2.2. Plant collection

M. ovalifolia leaf samples were collected from four locations in Namibia as follows: Rocky Hills in Moringa Safari Farm about 60 km from Okahandja to Karibib on a D2156 route in Otjozondjupa Region in central Namibia (Site 1), Sprokieswoud (Enchanted Forest), west of Okaukuejo in Etosha National Park (Site 2), Dolomite Hills near Halali in Etosha National Park (Site 3) and 3 km south of Tsumeb along B1 road side (Site 4). Respective herbarium specimens with the following voucher numbers: MNT 01, MNT 02, MNT 03 and MNT 04 representing plants from the four different sites were prepared and stored at the University of Namibia. A preliminary study to determine any major chemical differences between these was carried out and no significant differences were obtained.

2.3. Metabolites extraction

The *Moringa* leaves were air-dried and crushed using a pestle and mortar at room temperature to a fine-coarse powder with a relatively homogeneous particle size to make a composite sample. Metabolites were extracted from a homogeneous sample consisting of equal plant materials from the four different sites. Mixed ground leaves (2 g) were mixed with 80% aqueous methanol (MeOH) (20 mL) by means of sonicating for 30 min using an ultrasonic water bath (SB-120DT, Loyal Key Group (Hong Kong) Co. Ltd). To remove the tissue debris, the homogenates were centrifuged at 5000 × *g* for 10 min. The supernatant was concentrated under low pressure using a rotary evaporator at 55 °C. The resulting concentrate was transferred to a 2 mL Eppendorf tube and dried to completeness using the speed vacuum concentrator centrifuge at 55 °C. The resulting pellet was re-constituted to 1.0 mL by redissolving in 50% MeOH and filtered through 0.22 μm nylon filters. The extracts were kept at −20 °C until analysed on the UPLC-qTOF-MS.

2.4. UPLC conditions

A Waters Acquity UPLC coupled to a SYNAPT G1 HDMS mass spectrometer was used to generate accurate mass data. The chromatographic separation of the extracts was accomplished on a Waters BEH C_8 column (150 mm × 2.1 mm, 1.7 μm) and with column temperature controlled at 60 °C. A binary solvent mixture was used consisting of water (eluent A) containing 10 mM formic acid (natural pH of 2.3) and acetonitrile (eluent B). The initial conditions were 98% A at a flow rate of 0.4 mL/min and were 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 and re-equilibrated for 3 min prior to the next injection. The total run time was 30 min and the injection volume was 1 μL.

2.5. MS conditions

The SYNAPT G1 mass spectrometer was used in V-optics and operated in electrospray mode to enable detection of phenolic compounds. Leucine enkephalin (50 pg/mL) was used as the reference calibrant to obtain typical mass accuracies between 1 and 5 mDa. The mass spectrometer was operated in negative ion mode with a 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 to 1000 Dalton mass range. The source temperature was 120 °C and the desolvation temperature was set at 450 °C. Nitrogen was used as the nebulisation gas at a flow rate of 700 L/h. The raw UPLC-MS data was extracted and analysed using Mass Lynx XS software (Waters Corporation, Manchester, UK).

The CGA were profiled by collecting MS/MS (typical MS^2) data of the masses of interest. CQA were monitored at *m/z* 353, pCoQA at *m/z* 337, FQA at *m/z* 367, and dicaffeoylquinic acids (*di*CQA) and CQA-glycosides at *m/z* 515. Accordingly, the optimization process made use of authentic standards: 3CQA, 4CQA, 5CQA, 3,5*di*CQA, 3,4*di*CQA and 4,5*di*CQA (Phytolab, Germany). Moreover, extracts of alcoholic cider and coffee, commodities in which the CGA have been thoroughly characterized by LC-ion trap MS (Clifford, 2003; Clifford et al., 2003, 2007; Jaiswal and Kuhnert, 2010; Kuhnert et al., 2012; Hussain et al., 2014) were also used to serve as excellent surrogate standards for the *trans* 3-acyl, 4-acyl and 5-acyl *p*CoQA, CQA, FQA and *di*CQA which they contain. This helped to validate the developed LC-ion trap MS method.

To achieve the fragmentation pattern of the various compounds reported in Clifford et al. (2003), the trap collision energy was increased (3–60 eV) to afford a stable fragmentation pattern and was optimized for each compound class. To further enable distinction between the CQA and *di*CQA, the cone voltage was raised (10–100 V) to enable the formation of the following stable ions: Q1[quinic acid-H]− at *m/z*

Table 1
Summary of the *m/z* ions (quasi-molecular ion peak, C_1, Q_1, C_2, Q_2 and others) used for the identification of CGAs in *M. ovalifolia* by UPLC-qTOF-MS/MS.

Molecule no.	Molecule identity	Rt (min)	Precursor ion (*m/z*)	Product ions (*m/z*)
1	*trans* 3-pCoQA	5.67	337.09	163.04;119.04
2	*trans* 4-pCoQA	7.96	337.09	173.04; 119.04
3	*trans* 5-pCoQA	7.62	337.09	191.05; 119.04
4	*trans* 3-CQA	4.61	353.09	191.05; 179.05; 135.04
5	*trans* 4-CQA	6.10	353.09	191.05; 135.04
6	*trans* 5-CQA	5.78	353.09	191.05; 135.04
7	*trans* 3-FQA	6.63	367.09	193.04
8	*trans* 4-FQA	9.55	367.09	173.04
9	*trans* 5-FQA	9.28	367.09	191.05
10	*cis* 3-pCoQA	5.54	337.09	163.04; 199.04
11	*cis* 4-pCoQA	7.26	337.09	173.04; 119.04
12	*cis* 5-pCoQA	10.58	337.09	191.05; 119.04
13	*cis* 3-CQA	4.58	353.09	191.05; 179.05; 135.04
14	*cis* 4-CQA	5.64	353.09	191.05; 179.05; 135.04
15	*cis* 5-CQA	8.32	353.09	191.05; 135.04
16	*cis* 3-FQA	6.63	367.09	193.04
17	*cis* 4-FQA	8.78	367.09	173.04
18	*cis* 5-FQA	12.49	367.09	191.05
19	3,5 *di-CQA*	6.30	515.13	353.08; 191.05; 135.04
20	3-CQA-glycoside	4.78	515.14	353.08; 179.02; 341.07; 135.04
21	4-CQA-glycoside isomer 1	5.18	515.12	353.08; 341.08; 179.02; 173.04; 135.04
22	4-CQA-glycoside isomer 2	5.47	515.12	353.08; 341.08; 323.06; 179.02; 173.04; 135.04