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Identification of hydroxylcinnamoyl tartaric acid esters in *Bidens pilosa* by UPLC-tandem mass spectrometry

B.S. Khoza [a], S. Gbashi [b], P.A. Steenkamp [a,c], P.B. Njobeh [b], N.E. Madala [a,*]

[a] *Department of Biochemistry, University of Johannesburg, P.O. Box 524, Auckland Park, 2006 South Africa*
[b] *Department of Biotechnology and Food Technology, Faculty of Science, University of Johannesburg, P.O. Box 17011, Doornfontein Campus, 2028 Gauteng, South Africa*
[c] *Council for Scientific and Industrial Research (CSIR), Biosciences, Natural Products and Agroprocessing Group, Pretoria 0001, South Africa*

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ABSTRACT

Bidens pilosa is a medicinal plant used for the treatment of several physiological illnesses. In South Africa, as the case may be for other African countries, this plant is equally consumed as a vegetable. In the current study, pressurized hot water extraction (PHWE) technology was employed for the extraction of polyphenolic compounds from leaves of *B. pilosa* under two different temperature conditions (100 and 150 °C). Accordingly, extraction of these compounds was made possible at 150 °C and analysis of these extracts using UPLC-qTOF-MS/MS revealed the presence of several hydoxylcinnamoyl tartaric acids. Here, different isomers of coutaric-, caftaric-, fertaric-, chicoric acid and caftaric acid glycosides were detected. The contribution of mass spectrometry fragmentation towards the characterization of these molecules is also presented. To the best of our knowledge, this is the first report of these molecules in *B. pilosa*.

1. Introduction

Bidens pilosa is a herbaceous, annual flowering plant within the Asteraceae family, with about 240 known species (Bairwa et al., 2010; Arthur et al., 2012). Originally from Southern America, it is widely distributed in all tropical and subtropical regions of the world (Tereza et al., 2006). Its common names are black-jack (South Africa), beggars tick (USA), cobblers peg, farmer's friend (Australia), and Spanish needle (Barbados) (Arthur et al., 2012). *B. pilosa* is an easy-to-grow, highly invasive and adaptive herb that is registered as a weed, and hence considered one of the most troublesome wild plants in East Africa (Arthur et al., 2012; Bartolome et al., 2013). In many African countries such as South Africa, Kenya, Zambia, Mozambique, and Zimbabwe, this plant is consumed as a vegetable or pot herb (Karis and Ryding, 1994). In traditional medicine, the plant is known to potentiate several biological activities such as anti-microbial (Farias and Mendez, 2014), anti-cancer and anti-pyretic (Sundararajan et al., 2006), anti-inflammatory and anti-allergic (Horiuchi and Seyama, 2008), anti-oxidative (Chiang et al., 2004), anti-diabetic (Chien et al., 2009; Lai et al., 2015) as well as other beneficial attributes reviewed in studies (Bairwa et al., 2010; Bartolome et al., 2013).

The above-mentioned bioactivities could be due to a wide spectrum of phytochemicals present in this plant (Bartolome et al., 2013). Different parts of the plant have been shown to possess pharmacological properties (Oliveira et al., 2004; Yuan et al., 2008; Ashafa and Afolayan, 2009; Kviecinski et al., 2011). Several compounds have been identified in this plant and these include polyacetylenes and flavonoids which are the predominant classes of metabolites in the *Bidens* genus (Silva et al., 2011). In addition, other phenolic compounds such as caffeoylquinic acid derivatives (Chiang et al., 2004), polyacetylenic compounds (Tobinaga et al., 2009) and sesquiterpenes (Grombone-Guaratini et al., 2005) have also been characterized as chemical constituents of *B. pilosa*. Phenolic compounds such as hydroxycinnamoyl esters (e.g. hydroxycinnamoyl tartaric acids) are accumulated by plants to protect them against biotic and abiotic stresses (Sullivan, 2014). These compounds are known to possess several biological characteristics such as those presented above, especially the anti-viral activities (Chhipa et al., 2014). In the current study, we report for the first time the presence of hydroxycinnamoyl tartaric acids in *B. pilosa*. Here, a pressurized hot water extraction (PHWE) technology was applied for the extraction of hydroxycinnamoyl tartaric acid from air-dried *B. pilosa* leaves. Aspects of MS characterization of different isomers of hydroxycinnamoyl tartaric acid molecules detected in *B. pilosa* are also presented herein for future references.

2. Materials and methods

2.1. Plant material and metabolite extraction

B. pilosa leaves were collected from different villages of the Venda region of the Limpopo province, South Africa. Voucher herbarium

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[*] Corresponding author. Tel.: +27 11 559 4573; fax: +27 11 559 2605.
E-mail address: emadala@uj.ac.za (N.E. Madala).

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specimens (with voucher number NEM002) were prepared and deposited to the Department of Botany, University of Johannesburg. The leaves were air-dried at room temperature in a secluded area. Metabolites from the dried leaves (3 g) were extracted with pure water (pH 6.8) using a homemade pressurized hot water extraction system (Khoza et al., 2014) at 100 and 150 °C, at a constant pressure of 1000 ± 100 psi and flow rate of 2 mL/min. All extracts were filtered through a 0.22 μm membrane syringe filter into a glass vial and stored at 4 °C until use.

2.2. Chromatography and mass spectrometry analyses

Extracts (three technical replicates) were analysed using a UPLC coupled with a high definition Synapt G1 MS instrument using an Acquity HSS T3 C18 column (150 mm × 2.1 mm with particle size of 1.7 μm) (Waters, MA, USA). The mobile phase for the chromatographic analysis consisted of 0.1% formic acid in deionized water (Eluent A) and 0.1% formic acid in acetonitrile (Eluent B). The column was eluted with a linear gradient from 98% Eluent A to 5% Eluent A at 25 min while maintaining a constant flow rate of 0.4 mL/min, with a final column equilibration/wash for 3 min using 5% of Eluent B. This 30 min chromatographic elution was monitored by both photodiode array (PDA) and MS detectors. The photodiode array (PDA) detector scanning range was set from 200 to 500 nm, with 1.2 nm resolution and a sampling rate of 20 points/sec. For MS, data was acquired in a negative ionization mode using optimized settings presented elsewhere (Ncube et al., 2014). Alternating collision energies (CE) (3, 10, 20 and 30 eV) were utilized to achieve useful MS fragmentation data.

3. Results and discussion

Extraction plays a key role in the analysis of phytochemicals in plant materials (Özel and Gögüs, 2014). Pressurized hot water extraction (PHWE) compares favourably over conventional solvent-based extraction methods (Hartonen et al., 2007). In fact, it is suitable for the extraction of different phytochemicals (Liu et al., 2013), i.e., phenols, anilines and flavones from orange peels (Lamm and Yang, 2003), chlorogenic acids and flavonoids from *Moringa oleifera* leaves (Khoza et al., 2014) and anthraquinones from roots of *Morinda citrifolia* (Shotipruk et al., 2004). In the current study, PHWE was used to extract a wide spectrum of metabolites from a weed plant, *B. pilosa*. Based on the two temperature conditions, i.e., 100 and 150 °C set on the instrument, it was only possible to extract the above mentioned metabolites at 150 °C using water as an extraction solvent. Subsequently, a partially targeted UPLC-qTOF-MS approach (Madala et al., 2014; Ncube et al., 2014) was followed for the characterization of hydroxycinnamoyl esters as seen from the full scan chromatogram (Fig. 1). However, identification of these compounds was not effective using the full scan MS method and, as such, a robust and sensitive tandem MS approach was carried out (Fig. 2) using settings presented elsewhere (Ncube et al., 2014). From the MS fragmentation patterns (Figs. 3 & 4), the data shows that several cinnamic acid derivatives harbouring tartaric acid were identified. Accordingly, molecules **1–5** (Fig. 2, Table 1) were tentatively identified to be the isomeric (regional and geometrical) molecules of hydroxycinnamoyl tartaric acids. The presence of these molecules in several Asteraceae plants has been reported (Jaiswal et al., 2011) and their MS fragmentation presented elsewhere (Buiarelli et al., 2010; Jaiswal et al., 2011; Farias and Mendez, 2014). Based on the individual molecular fragmentation patterns (Figs. 2 & 3), all these molecules were positively identified.

Molecule **1** at retention time (Rt) of 9.964 min (Fig. 2D) produced a precursor ion at *m*/*z* 473.0905 $[M - H]^-$ ($C_{19}H_{22}O_{14}$) and fragmentation of this molecule (Fig. 4) generated product ions at *m*/*z* 341 (caffeoyl hexose), derived from the loss of a tartaric acid (132 Da). It also produced product ions at *m*/*z* 179 (caffeic acid) due to the neutral loss of 162 Da (hexose) and at *m*/*z* 135 (decarboxylated caffeic acid). Based on these data, molecule **1** was tentatively identified as caftaric acid hexose. Interestingly, molecule **2** at Rt of 10.072 min also showed the same fragmentation pattern (Fig. 4) as molecule **1** and, as such, these two molecules were identified as either geometrical or regional isomers of caftaric acid hexose. To the best of our knowledge, there is no single MS report of the caftaric acid hexose in literature, however, a similar fragmentation pattern has been noted elsewhere (Schütz et al., 2005). The chromatographic elution of molecules **1** and **2,** as seen in Fig. 2D, is also an indication that these two have very similar structures and polarities resulting in partial co-elution. In future, it will be best to distinguish between the two molecules with a more robust MS method, with different MS conditions to distinguish between similar caffeoyl glycosides (Ncube et al., 2014). In a separate study, ten different isomers of caffeoylglycosides were distinguished based on the underlying differences in fragmentation patterns which arose due to different capabilities of forming intra-molecular hydrogen bonding (Jaiswal et al., 2014). These results also show that caftaric acid glycoside (molecule **1**) and

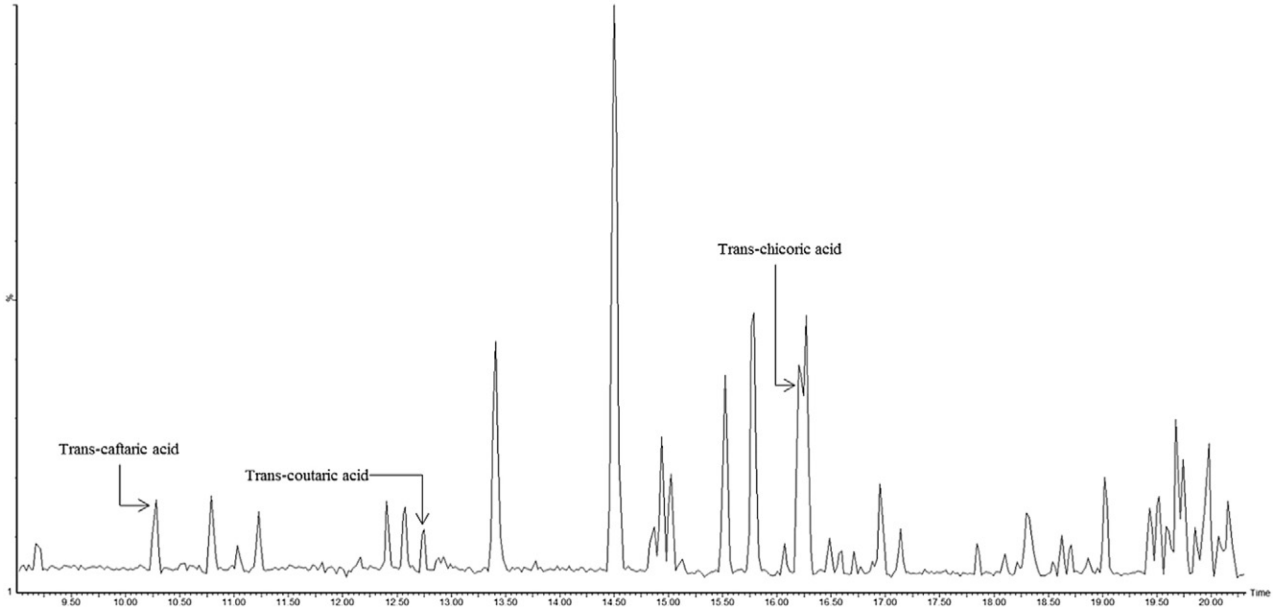

Fig. 1. Representative UPLC-qTOF-MS base peak ion (BPI) chromatogram showing the elution and relative intensities of some of the detected compounds.