Phytochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

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

# UHPLC-PDA-ESI-TOF/MS metabolic profiling of *Arctostaphylos pungens* and *Arctostaphylos uva-ursi*. A comparative study of phenolic compounds from leaf methanolic extracts

Alessia Panusa [a,*], Rita Petrucci [b], Giancarlo Marrosu [b], Giuseppina Multari [a], Francesca Romana Gallo [a]

[a] Dipartimento del Farmaco, Istituto Superiore di Sanità, Viale Regina Elena 299, I-00161 Rome, Italy
[b] Dipartimento di Scienze di Base e Applicate per l'Ingegneria SBAI, Sapienza Università di Roma, Via del Castro Laurenziano 7, I-00161 Rome, Italy

ARTICLE INFO

*Article history:*
Received 28 May 2014
Received in revised form 20 November 2014
Available online xxxx

*Keywords:*
*Arctostaphylos pungens*
*Arctostaphylos uva-ursi*
Ericaceae
Bearberry
UHPLC-PDA-ESI-TOF/MS
Arbutin
Gallotannins
Flavonols
Myricetin
Quercetin

ABSTRACT

The aim of this study was to get a rapid metabolic fingerprinting and to gain insight into the metabolic profiling of *Arctostaphylos pungens* H. B. K., a plant morphologically similar to *Arctostaphylos uva-ursi* (L.) Spreng. (bearberry) but with a lower arbutin (Arb) content. According to the European Pharmacopoeia the Arb content in the dried leaf of *A. uva-ursi* (L.) Spreng. must be at least 7% (wt/wt) but other species, like *A. pungens*, are unintentionally or fraudulently marketed instead of it. Therefore, methanolic leaf extracts of nine *A. uva-ursi* and six *A. pungens* samples labeled and marketed as "bearberry leaf" have been analyzed. A five-minute gradient with a UHPLC-PDA-ESI-TOF/MS on an Acquity BEH $C_{18}$ (50 × 2.1 mm i.d.) 1.7 μm analytical column has been used for the purpose. A comprehensive assignment of secondary metabolites has been carried out in a comparative study of the two species. Among twenty-nine standards of natural compounds analyzed, fourteen have been identified, while other fifty-five metabolites have been tentatively assigned. Moreover, differences in both metabolic fingerprinting and profiling have been evidenced by statistical multivariate analysis. Specifically, main variations have been observed in the relative content for Arb, as expected, and for some galloyl derivative like tetra- and pentagalloylglucose more abundant in *A. uva-ursi* than in *A. pungens*. Furthermore, differences in flavonols profile, especially in myricetin and quercetin glycosilated derivatives, were observed. Based on principal component analysis myricetrin, together with a galloyl arbutin isomer and a disaccharide are herein proposed as distinctive metabolites for *A. pungens*.



## 1. Introduction

*Arctostaphylos uva-ursi* (L.) Spreng. (Ericaceae) commonly known as uva ursi or bearberry, is a medicinal plant used since the 2nd century which flourishes in humus-rich soils of Europe, North America, Siberia, Iberian Peninsula, and Asia, in particular in the Himalayas (Naczk et al., 2011). The bearberry name derives from the edible fruit enjoyed by bears. The active substance in *A. uva-ursi* is arbutin (4-hydroxyphenyl-β-D-glucopyranoside, Arb) a natural phenolic compound widespread in vegetal species. It is found in the leaves of cranberry and blueberry, in blueberry shrubs, in different types of pears and in many other plants (Cui et al., 2005). High levels of Arb content in plants can be related to adaptation to stress conditions, like arctic low temperatures (*Vaccinium* spp., *Arctostaphylos* spp.) or drought stress (*Myrothamnus flabelifolia* Welw.) (Pop et al., 2009).

Different pharmacological properties of Arb have been investigated i.e., antimicrobial (Cybulska et al., 2011), antioxidant (Pavlović et al., 2011), anti-inflammatory (Lee and Kim, 2012), anti-hyperglycaemic and antihyperlipidemic (Shahaboddin et al., 2011). The use of bearberry leaf is recommended in the treatment of the urogenital tract infections since the in vivo hydrolyzed form, hydroquinone, is responsible for antiseptic and astringent effects (Glockl et al., 2001). Arb is also used in cosmetics as skin-whitening agent because of its potent tyrosinase inhibitory activity which suppresses melanin biosynthesis in human skin (Chisvert et al., 2010).

Other main constituents of *A. uva-ursi* are gallotannins (Pegg et al., 2008; Slaveska-Raicki et al., 2003) molecules which contain a sugar core, esterified either with one or several gallic acid units. Further gallic acid units can also be attached through a depside bond (Tian et al., 2009). The antioxidant (Cai et al., 2004; Tian et al., 2009), anti-inflammatory (Erdelyi et al., 2005), anticancer (Erdelyi et al., 2005; Kuo et al., 2009), anti-diabetic and cardio protective (Lee et al., 2010) properties of such molecules have been described. Moreover, a great number of other secondary metabo-

* Corresponding author. Tel.: +39 06 49902093; fax: +39 06 49387100.
*E-mail address:* alessia.panusa@iss.it (A. Panusa).

http://dx.doi.org/10.1016/j.phytochem.2015.01.002
0031-9422/

lites like flavonols, flavanols, anthocyanins, procyanidins, hydroxycinnamic acids have been described to act synergistically (Eugster et al., 2011; He, 2000) and are also considered as useful chemotaxonomic markers (Alonso-Salces et al., 2004; Ducrey et al., 1995).

In the past, some bearberry adulterations have been reported for European cowberry (*Vaccinium vitis-idaea* Linné) and other Mexican Ericaceae spp. (e.g., *A. tomentosa* Douglass, *A. mucrocifera* De Candolle, *A. polifolia* Kunth, *Arbutus xalapensis* Kunth and *V. uliginosum* Linné) (Applequist, 2006; Mills and Bone, 2005). More recently, it has been evidenced that other species of the *Arctostaphylos* genus with an Arb content in dried leaf considerably lower than 7% (wt/wt), like *Arctostaphylos pungens*, are marketed in place of *A. uva-ursi* since the two species are morphologically similar (Gallo et al., 2013). In the "bearberry leaf" monograph of the European Pharmacopoeia (Council of Europe, 2014) an Arb minimum content of 7% (wt/wt) in dried leaf is required, while products containing a lower content of Arb can be marketed as food supplements.

Constituents of *A. uva-ursi* have been extensively investigated in the past especially by TLC and HPTLC (Braz et al., 2012; Slaveska-Raicki et al., 2003) and recently by hyphenated techniques like HPLC-MS (Olennikov and Chekhirova, 2013; Saleem et al., 2010). To the best of our knowledge there are neither a UHPLC-PDA fingerprinting nor a UHPLC-TOF/MS metabolic profile for *A. uva*-ursi or *A. pungens* although anti-lipase and antioxidant properties of this latter have recently been investigated (Villa-Ruano et al., 2013). The use of a state-of-the-art instrumentation which combines ultra-high pressure liquid chromatography (UHPLC) with high-resolution accurate mass measurement, permits the separation of a great number of metabolites in few minutes compared to conventional HPLC-MS. Moreover, principal component analysis (PCA) evidences metabolites which discriminate one species from one another. Hence, UHPLC-PDA-ESI-TOF/MS analyses of dried leaf methanolic extracts have been carried out on fifteen different commercially available samples, labeled and marketed as "bearberry leaf" but previously recognized as belonging either to *A. uva-ursi* or *A. pungens* species (Gallo et al., 2013). Consequently, the aim of this work was firstly, to carry out a multivariate analysis based on PCA in order to evidence differences in metabolic profiling of *A. pungens* and *A. uva-ursi* and then to get a fingerprinting for a rapid identification of the same species. Secondly, to perform a comprehensive comparative study on metabolic profiling for both species in order to gain insight into the chemical composition of *A. pungens*. A particular attention was paid to polyphenols and gallotannins with the aim to find a potential marker to distinguish *A. pungens* from *A. uva-ursi*. Finally, to evaluate the variation in the content of Arb and other main components in the two species.

A number of isomers of gallotannins as well as flavonols that have not been previously reported for *A. uva-ursi* have been evidenced. The assignment of fourteen identified compounds and the putative assignment of other fifty-five compounds (Creek et al., 2014; Sumner et al., 2007) have been carried out. Similarities and differences in qualitative and quantitative composition of the two species have been pointed out and characteristic metabolites for each species have been evidenced. Furthermore, a comprehensive metabolic profile of *A. pungens* has been outlined for the first time.

## 2. Results and discussion

### 2.1. UHPLC analysis

As comprehensively demonstrated (Eugster et al., 2011; Grata et al., 2009) the use of UHPLC has made possible to shorten significantly the analysis time of multi-component analytes and to increase peak capacity, sensitivity and reproducibility compared to HPLC. In this study, a five-minute gradient was used to elute all main phenolic constituents of methanolic leaf extracts; in fact, no other peaks were detected beyond the first 6 min of the analysis.

Good separation of peaks was achieved between myricetin (Myr) and quercetin 3-rhamnoside that were not completely separated by Saleem et al. (2010) with a twenty-five-minute gradient. An excellent reproducibility of retention time ($R_t$) was obtained, variability being always not higher than 0.02 min.

### 2.2. Principal component analysis

PCA gave rise to a two-component solution explaining 93% of total variance (Supplementary Table S1). PC1 (60%) has all positive loadings whose values are all higher in *A. uva-ursi* than in *A. pungens* (Supplementary Table S2). It reveals that the variation of metabolites in different samples goes in the same direction or, in other words, the relations among different peak areas are common across all samples. PC2 (33%) perfectly discriminates *A. uva-ursi* from *A. pungens* samples with *A. uva-ursi* having all negative and *A. pungens* all positive loadings for all samples (Supplementary Table S2). Both PC1 and PC2 discriminate the two species as evidenced in Fig. 1 where the loadings space of *A. uva-ursi* and *A. pungens* profiles is depicted. In particular, this figure undoubtedly shows that samples 1, 3, 4, 7, 9, 10, 12, 13, 14 belong to one species (*A. uva-ursi* L.) while the other six samples (2, 5, 6, 8, 11, 15) to one other (*A. pungens* H. B. K.). The most discriminating metabolites are those with a score higher than 2 in modulus (corresponding to 2 s.d. from component mean, data not shown) for both PC1 and PC2. In PC1 they correspond (see Table S3) to arbutin **3** ($R_t$ = 0.62 - min, score 4.90), tetragalloylglucose **59** ($R_t$ = 3.34 min, score 4.51) and pentagalloylglucose **78** ($R_t$ = 3.82 min, score 2.39) with peak areas more abundant in *A. uva-ursi* than in *A. pungens*. In PC2 they correspond to metabolites characteristic in *A. pungens*, i.e., disaccharide **11** ($R_t$ = 1.17 min, score 4.47); galloylarbutin **29** ($R_t$ = 2.23 - min, score 2.85); myricetrin **61** ($R_t$ = 3.34 min, score 2.75). There are also few metabolites in PC2 with a score less than 2 s.d. but not negligible i.e., myricetinhexoside **53** ($R_t$ = 3.01 min, score 1.8) and galloylshikimicacid **17** ($R_t$ = 1.58 min, score 1.4).

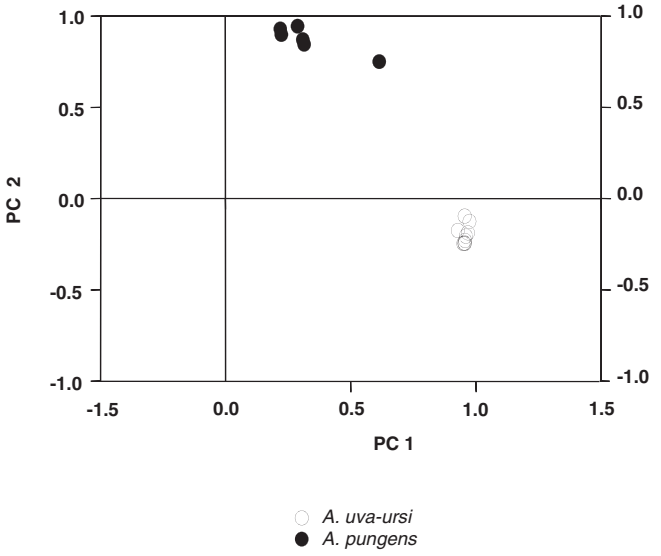


**Fig. 1.** Differences between *A. uva-ursi* and *A. pungens* profiles in the component PC1 and PC2 loadings space.

Please cite this article in press as: Panusa, A., et al. UHPLC-PDA-ESI-TOF/MS metabolic profiling of *Arctostaphylos pungens* and *Arctostaphylos uva-ursi*. A comparative study of phenolic compounds from leaf methanolic extracts. Phytochemistry (2015), http://dx.doi.org/10.1016/j.phytochem.2015.01.002