Industrial Crops & Products 108 (2017) 44–51

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Profile of bioactive secondary metabolites and antioxidant capacity of leaf exudates from eighteen *Aloe* species

Mariateresa Cardarelli[a], Youssef Rouphael[b], Marco Pellizzoni[c], Giuseppe Colla[d], Luigi Lucini[c,*]

[a] Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria, Centro di ricerca per lo studio delle Relazioni tra Pianta e Suolo, Rome, Italy
[b] Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy
[c] Institute of Environmental and Agricultural Chemistry, Università Cattolica del Sacro Cuore, Piacenza, Italy
[d] Department of Agricultural and Forestry Sciences, University of Tuscia, Viterbo, Italy

ARTICLE INFO

Keywords:
Chromones
High-resolution MS
Phytochemical profiling
Secondary metabolites
Xanthorrhoeaceae

ABSTRACT

Over the past years, interest in the phytochemical profile of *Aloe* species (family: *Xanthorrhoeaceae*) has been on the rise, compelled by its popularity as an ingredient in cosmetic formulations, food supplements and drug design. It is surprising that only few *Aloe* spp. have been grown and commercialized; this might be due to the very limited knowledge of their potent bioactive compounds. The phytochemical profile of eighteen *Aloe* species was investigated using colorimetric assays, triple quadrupole and time-of-flight mass spectrometry, focusing on bioactive secondary metabolites in leaf exudates. The phytochemical profile of leaf exudates from different species was widely diverse and included free and glycosylated chromones (mainly aloeresins, with a content of aloeresin A up to 843.4 g 100 g^{-1}) followed by the anthraquinones aloin (0.66–4.96 g 100 g^{-1}) and hydroxyaloins. Among the examined species *A. marlothii*, and *A. melanacantha* were found to be richest in total polyphenols (14.3 and 6.4 g gallic acid equivalents 100 g^{-1}), flavonoids (up to 7.1 g rutin equivalents 100 g^{-1}), flavonols (up to 5.0 g rutin equivalents 100 g^{-1}), and presented the highest antioxidant activity (up to 810.3 μmol g^{-1} trolox for DPPH, and 453.4 μmol g^{-1} trolox for ORAC), aloin, and aloeresin A contents. A second cluster, including *A. arborescens* and *A. nyeriensis,* was characterized by high contents of total phenolics and aloenin. These findings highlight for the first time the complex profile of phytochemicals in aloe leaf exudates and open new horizons to the industrial use of these *Aloe* species, which could represent a smart approach to increase the growers' income.

1. Introduction

The genus *Aloe* (family: *Xanthorrhoeaceae*) comprises over 500 species, ranging from small shrubs to tree-like plants (Grace, 2011). *Aloe* species are subtropical plants with succulent leaves traditionally used for wound healing in natural medicine (Cristiano et al., 2016). In recent years, *Aloe* has generated significant interest among consumers and researchers due to its nutraceutical substances extracted from leaves for alimentary, cosmetic and pharmaceutical industries (Grace et al., 2008; Chen et al., 2012; Amoo et al., 2014; Javed and Atta-Ur, 2014; Cristiano et al., 2016). In numerous pre-clinical and clinical studies the *Aloe* gel has exhibited various pharmacological activities and therapeutic effects such as anti-inflammatory, antimicrobial, antiplasmodial, antimalarial, antioxidant and anticancer activity (Grace et al., 2008; Lucini et al., 2015a). It has been postulated that these biological properties of *Aloe* extracts can be ascribed to the synergistic action of several phytochemicals and antioxidants (*e.g.*, phenolics, polysaccharides and vitamins) (Romani et al., 2008; Pellizzoni et al., 2012a; Lucini et al., 2015a). Among phenolic compounds, aloin was widely studied as one of the main biologically active components of *Aloe* exudates (Liao et al., 2006; Javed and Atta-Ur, 2014). Similarly, other chromones, anthraquinones and anthrones were often reported to form the profile of phenolic compounds in *A. barbadensis*, *A. arborescens* and in other lesser-known *Aloe* species (Bisrat et al., 2000; Kuzuya et al., 2001; Chen et al., 2012; Grace et al., 2013; Lucini et al., 2013).

The quantitative and qualitative variation of bioactive phytochemicals in leaf mesophyll commonly known as *Aloe* gel and/or exudates depends upon several pre-harvest factors such as genetic material, fertilization, salinity and the environment during the plant growth (Cardarelli et al., 2013; Zapata et al., 2013; Amoo et al., 2014; Cristiano et al., 2016). However, genetic material is a key pre-harvest factor and the major determinant of variation in the level of bioactive compounds of *Aloe* gel (Amoo et al., 2014).

Despite the great variability in the *Aloe* genus, only few species

* Corresponding author at: Institute of Environmental and Agricultural Chemistry, Università Cattolica del Sacro Cuore, Via Emilia Parmense, 84-29122 Piacenza, Italy.
E-mail address: luigi.lucini@unicatt.it (L. Lucini).

http://dx.doi.org/10.1016/j.indcrop.2017.06.017
Received 19 November 2016; Received in revised form 15 May 2017; Accepted 6 June 2017

[mainly *Aloe vera* L. (A. *barbadensis* Mill.) and *Aloe arborescens* Mill.] are currently used for the extraction of cosmetic and nutraceutical active ingredients. Datamonitor Consumer's Product Launch Analytics database reported that *A. vera* was the only *Aloe* species used as ingredient in more than 50 different product categories [non-food categories (*i.e.*, soap, products for facial care, bath and shower) and juices and functional drinks] since 2012 (Steenkamp, 2015). In view of the broad number of *Aloe* species, it is surprising that only a few species have been grown and commercialized; this might be due to the limited knowledge of the phytochemical potential across species. In this sense, the identification and quantification of bioactive compounds in *Aloe* genus could allow the exploitation of the enormous potential locked up in this genus for the cosmetic, pharmaceutical and food industries.

In a previous study, Zapata et al. (2013) studied the gel chemical composition from different *Aloe* species at three growing seasons (winter, spring and summer). The previous work was focused on eight *Aloe* species (*A. arborescens* Mill., *A. aristata* Haw., *A. claviflora* Burch, *A. ferox* Mill., *A. mitriformis* Haw., *A. saponaria* Haw., *A. striata* Haw., and *A. vera* L.) without considering many *Aloe* species of potential interest for industrial use. Sánchez-Machado and co-workers (Sánchez-Machado et al., 2017) recently reviewed the chemical composition of *Aloe* mainly focusing on carbohydrate profile of leaves, while Zapata et al. (2013) focused on target compounds (aloin, antioxidant activity, polyamines, total lipids, proteins and phenolics), with limited information on the full secondary metabolites and antioxidant profile. In this regard, metabolomics have proven to be a novel and powerful tool for understanding biochemical networks involved in cultivar comparison as well as screening and quantifying the profile metabolites in a single analysis (Fukushima and Kusano, 2013; Farag et al., 2014; Lucini et al., 2015b, 2016a).

The objectives of the present study were: (1) to depict the antioxidant, target and untargeted secondary metabolites in leaf exudates from eighteen *Aloe* species grown under greenhouse conditions, and (2) to elucidate the associations between these bioactive traits using a combination of targeted and untargeted phytochemical profiling. With several *Aloe* species profiled for the first time, the results provide insight into chemical leaf exudates variation of a broad collection of *Aloe* that includes both marketed and new species.

2. Materials and methods

2.1. Plant material, growth conditions and sampling

Eighteen *Aloe* species, supplied by the Botanical Garden of Tuscia University, Viterbo, central Italy, (lat. 42°25′N, long. 12°08′E, alt. 310 m above sea level) were included in this study. The *Aloe* species were: *A. arborescens* Mill., *A. aristata* Haw., *A. barbadensis* Mill., *A. claviflora* Burch, *A. dichotoma* Masson, *A. erinacea* D.S. Hardy, *A. ferox* Mill., *A. grandidentata* Salm-Dyck, *A. marlothii* A. Berger, *A. melanacantha* A. Berger, *A. nyeriensis* Christian & I. Verd., *A. perfoliata* L., *A. plicatilis* (L.) Mill., *A. ramosissima* Pillans, *A. spinosissima* Hort., *A. squarrosa* Baker ex Balf.f, *A. striata* Haw., and *A. variegata* L.

Six-year old *Aloe* plants were grown under greenhouse conditions on sandy clay loam soil (66% sand, 19% silt and 15% clay), with a pH of 7.1, electrical conductivity of 0.3 dS m^{-1}, organic matter of 1.7% (w/w), total N at 0.1%, available P at 25 mg kg^{-1}, and exchangeable K at 3546 mg kg^{-1}. Plants were regularly watered as needed: from a minimum of once a month during winter season to a maximum of once a week during summer season. Annual fertilization was adopted during spring season (April) by applying 10 g $plant^{-1}$ of a 4N-1.7P–3.3K dry pelleted poultry fertilizer (Italpollina S.p.A, Rivoli Veronese, Italy). Fertilizer was scattered around each plant and immediately buried. Weeds were controlled through hand pulling and hand hoeing. No pesticides were sprayed during the growing cycle. On 12 June 2012, exudates were obtained by cutting the mature leaves at the base allowing the juice to drain out. All samples were instantly frozen in liquid nitrogen and stored at −80 °C until used.

2.2. Chemicals and reagents

Analytical grade pure compounds, the free radical DPPH·(1,1-diphenyl-2-picrylhydrazyl), the azo free radical generator AAPH (2,2′-azobis-2-amidinopropane dihydrochloride), fluorescein, and solvents (methanol and acetonitrile LCMS grade, acetone and ethyl acetate reagent grade) were purchased from Sigma Chemicals (St. Louis, MO, USA). Aloin, aloe-emodin, aloeresin A, aloesin, aloesone and aloenin were pure compounds kindly donated by the Department of Chemistry, University of Milan (Italy).

2.3. Preparation for assays

Each exudate was dissolved in a methanol, acetone and ethyl acetate mixture (70:20:10, v/v) under sonication for 8 min, centrifuged (2000*g*) for 20 min, and then the clear supernatant was removed and stored at −30 °C. Diluted solutions, each of them having a concentration of 300 mg of solids L^{-1} were prepared in methanol 80% and immediately stored at −30 °C for further use.

Aloin, aloe-emodin, aloeresin A, aloesin, aloesone and aloenin reference standards were prepared in methanol as stock solutions with final concentrations of 300 mg L^{-1}, sonicated for 10 min and stored in dark and closed tubes at −30 °C.

2.4. Total phenolic, flavonoids and flavonols content

Total phenolics, flavonoids and flavonols were determined as previously described (Lucini et al., 2015a; Borgognone et al., 2016). Total phenolic compounds were estimated in each extract by the Folin-Ciocalteu assay. Aliquots (0.3 ml) of the extract were mixed with 0.7 ml of Folin-Ciocalteu reagent (Sigma, diluted five-fold) and 1.2 ml (75 g L^{-1}) sodium carbonate. Solutions were kept at 20 °C for 40 min in the dark and then absorbance was recorded at 765 nm. A calibration curve was prepared using aliquots of gallic acid ethanolic solutions and the results expressed as gallic acid equivalents (GAE).

Flavonoid content was determined from aliquots (0.1 ml) of the extract mixed with 0.1 ml $AlCl_3$ in ethanol (20 g L^{-1}) and diluted with ethanol to 2.5 ml. The resulting solution was kept at 20 °C for 40 min and absorption measured at 415 nm. Flavonols were measured by mixing 0.2 ml of extracts with 0.2 ml $AlCl_3$ (20 g L^{-1}) and 0.6 ml sodium acetate (50 g L^{-1}), then measuring absorbance at 440 nm after incubation at 20 °C for 2.5 h. A rutin calibration curve was used also for both flavonoids and flavonols, and their content expressed as rutin equivalents (RE).

2.5. Assay of antioxidant capacity

The antioxidant capacity of the leaf exudates was assessed as previously reported (Lucini et al., 2016b; Rouphael et al., 2016). The DPPH (2,2-diphenyl-L-picrylhydrazyl) as well as the oxygen-radical absorbing capacity (ORAC) radical scavenging activities, were determined.

Briefly, DPPH radical scavenging was determined from 1 ml of extract, placed in a cuvette with 1.5 ml of a 1.0×10^{-4} mol l^{-1} daily prepared ethanol solution of DPPH, then monitoring absorbance at 517 nm using a Perkin Elmer (Ontario, Canada) Lambda 12 spectrophotometer. However, ORAC capacity was carried out through fluorimetry using 2,2′-azobis-2-amidinopropane dihydrochloride (AAPH, from Sigma Aldrich) as peroxyl radical generator and fluorescein (Invitrogen, Monza, Italy) as a probe. A Synergy HT Multi-Detection Microplate Reader (BioTek Instruments, Inc. Winooski, VT), working at 485 nm for excitation and at 528 nm for emission, was used for this purpose. The wells in the plate were filled with 150 µl of fluorescein solution and then 25 µl of extract were added; the resulting solution was incubated for 30 min. Thereafter, reactions were initiated by