C. R. Chimie 19 (2016) 1124–1132

Contents lists available at ScienceDirect

Comptes Rendus Chimie

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A dereplication strategy for the identification of new phenolic compounds from *Anvillea radiata* (Coss. & Durieu)

Meryem Alaoui Boukhris [a, b], Émilie Destandau [a, *], Ahmed El Hakmaoui [b], Lhoucine El Rhaffari [c], Claire Elfakir [a]

[a] Université d'Orléans, CNRS, ICOA, UMR 7311, 45067 Orleans, France
[b] Université Hassan-II – Mohammedia-Casablanca, Laboratoire de Chimie Physique et Chimie Bioorganique, 28800 Mohammedia, Morocco
[c] Université Moulay-Ismail, Équipe de Recherche Environnement et Santé, Errachidia, Morocco

ARTICLE INFO

Article history:
Received 5 February 2016
Accepted 23 May 2016
Available online 30 June 2016

Keywords:
Anvillea radiata (Coss.&Durieu)
Flavonoids
Phenolic acids
PLE
HPLC-ESI/MS/MS
HPLC-HRMS

ABSTRACT

Phenolic compounds were selectively extracted from aerial parts of *Anvillea radiata* using Accelerated Solvent Extraction (ASE) in two steps. Given the two molecular families (flavonoids and germacranolides) described as present in the plant material, a first extraction step using chloroform as the extraction solvent was carried out to remove the germacranolides, the most abundant compounds. The minor phenolic compounds were then selectively extracted and enriched from the plant residue by methanol. Characterization of twenty five phenolic compounds in the methanolic extract was performed using HPLC-DAD-ESI-MS/MS and HPLC-HRMS analyses. Seven compounds corresponded to chlorogenic acid and dicaffeoylquinic acid derivatives and eighteen flavonoids (from which five aglycones and thirteen glycosides) were identified and some of them for the first time.
The presence of these phenolic compounds, identified in the whole aerial parts, was then followed in each organ (flower, leave and stem). The chromatographic profiles of the stem and leave were very close, while the flower one was more different. However most of the compounds identified in aerial parts were recovered in each organ, mainly difference on peak intensity could be observed. The most abundant compound in flowers was found to be a di-caffeoylquinic acid derivative while isorhamnetin and spinacitin diglucoside derivatives were the most abundant ones in stems and leaves.

1. Introduction

Anvillea garcinii subsp. radiata (Cosson & Durieu) is a wild plant from the Asteraceae family that grows predominantly in the steppes of North Africa (Morocco and Algeria) and found in areas of the Middle East. *Anvillea radiata* is a small woody shrub, densely branched, 20–50 cm high. The leaves are green-gray, small, and roughly triangular, with a large petiole and strongly toothed limb. The big solitary capitules have a diameter of 3–5 cm, with long ligules. The flowers are all yellow-orange, the outside one 25 mm long. It usually flowers in spring, but can flower throughout the year. It is widely used in traditional medicine for the treatment of dysentery, gastric-intestinal disorders, and chest cold [1] and has been reported to have hypoglycemic activity [2] as well as antifungal activity [3,4]. *A. radiata* has been previously reported in the literature to contain mainly germacranolide compounds. From different chloroform extracts of the aerial

* Corresponding author. Institut de Chimie Organique et Analytique, Université d'Orléans, UMR 7311, rue de Chartres, 45067 Orléans cedex 2, France.
E-mail address: emilie.destandau@univ-orleans.fr (É. Destandau).

http://dx.doi.org/10.1016/j.crci.2016.05.019

parts, six germacranolides have been isolated. They have been identified as 9α-hydroxyparthenolide [5] 9β-hydroxyparthenolide [6], 9β-hydroxy-1β,10α-epoxyparthenolide [6], 9α-hydroxy-1β,10α-epoxyparthenolide [7], 8α,9α-epoxyparthenolide [8], parthenolid-9-one [7] and cis-parthenolid-9-one [9]. Germacranolides showed anti-inflammatory and antitumor activities and presented a great interest [10].

A. radiata aerial parts also contain phenolic compounds that are less studied than germacranolides. Only two papers reported the presence of aglycone or glycoside flavonoid compounds in *A. radiata* [11,12] and the identification of all this molecular family is incomplete. Phenolic compounds are well known for their beneficial effects on human health and their ability to limit damage from oxidative stress due to radical species [13]. As a consequence, the aim of this work was to characterize the phenolic composition of *A. radiata* aerial parts in order to gain better insight into the molecular content of this plant and contribute to a better phytochemical knowledge and use of this wild indigenous plant with medicinal activity.

Precedent works [11,12] described long and fastidious multi-step methodologies using maceration, liquid-liquid extraction, purification by liquid chromatography (LC) on open columns, preparative LC and Thin Layer Chromatography (TLC) to purify and isolate some individual molecules before their identification as flavonoids by ^{1}H NMR. For some of them, less than 1 mg was lastly obtained making difficult their structural identification. Furthermore these methodologies were plant material, solvent and time consuming.

In the present work, we developed a simplified dereplication procedure to offer the possibility of obtaining structural information on all the phenolic constituents directly on-line. Selective extraction of flavonoids was carried out from *A. radiata* using accelerated solvent extraction (ASE) in two steps to remove, in a first step, major germacranolides present in the plant material and then to obtain from the plant residue free of germacranolides, an extract enriched in flavonoids. Rapid identification of compounds was then managed directly on the crude methanolic extract using HPLC-ESI/MS/MS and HPLC-High-Resolution MS (HPLC-HRMS) and without any need of further pre-treatment or prior compound isolation. This methodology was develop and applied to *A. radiata* aerial parts since the whole is frequently used in traditional medicine and would enter in cosmetic applications, then it was extend to each organ to qualitatively compare their phytochemical composition.

2. Materials and methods

2.1. Plant material

Aerial parts (flowers, leaves and stems) of *A. radiata* (Coss.&Durieu) were collected during the flowering period in May 2012 and May 2013, from Errachidia Road P21, Morocco. GPS coordinates were latitude (32.204086355917944) and longitude (−4.383201599121094). A voucher specimen has been deposited in the Herbarium of Scientific Institute, Rabat, Morocco.

The harvest parts were screened and free of contaminating portions then shade dried at room temperature. After drying, the plant material was stocked in the dark in the lab and was ground into fine powder using a basic grinder just before extraction.

2.2. Chemicals

Chloroform, methanol and formic acid were of analytical grade and provided by SDS Carlo Erba (Val-de-Reuil, France). Water was purified (resistance < 18 Ω) from ultrapure water using an Elgastat UHQ II system (Elga, Antony, France). The isorhamnetin, isorhamnetin-3-*O*-glucoside and chlorogenic acid were purchased from Extrasynthese (Genay, France).

2.3. Extraction procedure

An ASE 100 system from Dionex (Voisins le Bretonneux, France) with 34 mL stainless steel ASE vessels was used for the pressurized extraction. 2.5 g of powder material was mixed with Na_2SO_4 (2.5 g) as a dispersant agent and extracted successively with two different solvents (chloroform and then methanol). The following standard parameters recorded in ASE were applied: extraction time 5 min, flush volume of 65% and purge with nitrogen gas during 100 s at the end of each extraction. Extractions were carried out at 40 °C to avoid potential compound degradation with temperature and under a pressure of 100 bar. For each solvent two static cycles were performed. The liquid extracts (chloroform and methanol extracts) were then evaporated using a rotary evaporator (Buchi Labortechnik AG, Switzerland) under vacuum to obtain dried crude extracts before calculating an extraction yield.

2.4. Acid hydrolysis

Total acid hydrolysis of the methanol extract of aerial parts was carried out under the same conditions as reported in Ref. [14]. 1 mL of 4 M HCl was added to 1 mL of 10 g/L methanol extract solution. This mixture solution was kept in a closed vial for 30 min at 85 °C and directly analyzed by LC-DAD-ESI-MS/MS.

2.5. HPLC analysis

The HPLC system consisted of an Agilent Technologies Series 1100 system (Palto Alto, CA, USA) equipped with a binary pump. Chromatographic separations were performed on a Purospher® RP18e column (125 × 4 mm, 5 μm) (VWR Fontenay sous bois, France), at room temperature. The mobile phase was delivered at a flow rate of 1 mL min^{-1} and consisted of ultrapure water (solvent A) and methanol (solvent B) both acidified with 0.1% formic acid using gradient elution (0–40 min: 5–90% B, 40–50 min: 90% B). Equilibration time was of 10 min between two successive injections. The sample injection volume was 20 μL and the extract concentration was 1 mg/mL dissolved in the mobile phase. The detection was done with a UV-Visible Spectroflow 783 (Bristol, CT, USA) from Kontron Instruments (Montigny Le Bretonneux, France) set