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Short communication

# A re-investigation of the phytochemical composition of the edible herb *Amaranthus retroflexus* L.



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## ABSTRACT

In this paper the presence of selected prenylated and unprenylated phenylpropanoids of nutraceutical value, namely umbelliferone, apigenin, 4'-geranyloxyferulic acid, 7-isopentenyloxycoumarin, auraptene, and umbelliprenin have been determined in all parts of the edible herb *Amaranthus retroflexus* extracted with different methodologies. Roots were seen to contain the widest variety of unprenylated and prenylated phenylpropanoids both in terms of number of secondary metabolites and their quantitites. Findings described in the present study underline how *A. retroflexus* can be considered as a potential nutraceutical for human welfare.

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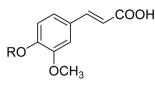
#### 1. Introduction

Amaranthus retroflexus L. (Amaranthaceae) is an annual herb. also commonly known as "pigweed", "redroot amaranth", and "wild beet". It usually grows up to 90 cm and has a frost tender consistency. The plant is native to the tropical Americas, but it is nowadays widespread in a vast number of habitats all over the world as an introduced species. All parts of A. retroflexus are consumed as food in several geographical areas. The first report indicate that Native Americans used this plant for medicinal purposes. Several tribes in fact believed that the whole plant mixed with wheat, honey, and white dove's hearth could have the power to win the love of whoever ate. Furthermore extracts of the aerial parts of A. retroflexus have been used as an astringent in case of profuse menstruation and as a magic herb during holy ceremonies [1]. Nowadays notable examples of consumption of A. retroflexus as food are those of Mexico, in which the whole dried plant is marketed as "Quelite quintonil", and of the Indian region of Kerala, where finely cut leaves mixed with grated coconut chilli peppers, garlic, and turmeric provide a popular dish called "thoran". Leaves of A. retroflexus have been seen to be a rich source of vitamins A and C, and iron. Seeds are eaten row or toasted. They can also be

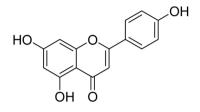
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http://dx.doi.org/10.1016/j.jpba.2017.05.051 0731-7085/© 2017 Elsevier B.V. All rights reserved. ground to give flours in turn used to prepare bread, consumed as an alternative to cereals, and as additives for salads. Finally aerial parts deprived of leaves and flowers are consumed as spinach in southern Molise region (Italy), where it is known with the popular name of "sghirro". Despite its wide use for culinary purposes, relatively few data on the phytochemical composition of A. retroflexus have been reported in the literature. Of more than 2900 references found in the Scifinder database about this plant, only 16 are focused on extraction, isolation, structural characterization, analysis, nutraceutical, and pharmacological properties of its primary and secondary metabolites. Indeed A. retroflexus is more known to be a powerful agronomic model, than a food supplement or as a medicinal plant. A. retroflexus was seen to be a source of fatty acids, in particular linoleic and linolenic ones [2], essential amino acids [3], and starch [4]. Secondary metabolites in so far isolated comprise flavonoids (e.g. rutin and quercetin) [5], alkaloids (e.g. amaranthine) [6], sesquiterpenes [7,8], phenolic acids [9], and volatiles [10]. Among the few biological data cited in the literature, Conforti and co-workers reported in 2011 that the extract of leaves of A. retroflexus showed an appreciable inhibition of nitric oxide release from LPS-stimulated RAW 264.7 macrophages ( $IC_{50} = 56.0 \mu g/mL$ ) [2]. These data clearly indicate that further studies are needed to depict in more details the phytochemical pool of A. retroflexus. Such investigations will be also of great help in better defining the nutraceutical and eventually commercial value of this plant. During the last decade we have been deeply involved in the characterization of the phytochemical profile of spontaneous edible herbs

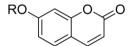
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1 R = H Ferulic acid 4 R = 3,3-dimethylallyl Boropinic acid 5 R = geranyl 4'-Geranyloxyferulic acid







2 R = H Umbelliferone 6 R = 3,3-dimethylallyl 7-Isopentenyloxycoumarin 7 R = geranyl Auraptene 8 R = farnesyl Umbelliprenin

Fig. 1. Structure of the secondary metabolites analyzed in A. retroflexus.

belonging to the Italian flora with a particular reference to the presence of biologically active oxyprenylated secondary metabolites. In this context we decided to investigate A. retroflexus for which, as stated above, a firm handed down oral tradition on its consumption as food exists in several regions of southern Italy. The aim of this work will be to identify and quantify selected secondary metabolites, some of which already described as component of other Amaranthus spp., in a wide number of extracts of roots, bark, leaves, and flowers obtained by different extraction techniques, namely maceration, ultrasound, and microwaves-assisted processes with increasing polarity solvents and partition with  $\beta$ -cyclodextrin ( $\beta$ -CD). More in particular secondary metabolites taken into consideration were ferulic acid **1**, a phenolic shown to be particularly abundant in Amaranthus spp. [9], umbelliferone 2, recently seen to be a component of roots and bark extracts of A. cruentus L. [11], apigenin **3**, found in polar extracts of A. cruentus and A. hispidus L. [12], and finally the prenylated derivatives of 1 and 2, boropinic acid 4 and 4'-geranyloxyferulic acid (GOFA) 5, 7-isopentenyloxycoumarin 6, auraptene 7, and umbelliprenin 8. These latters are described herein as components of the pyhtochemical pool of Amaranthus spp. for the first time. The structures of compounds 1-8 are illustrated in Fig. 1.

#### 2. Material and methods

#### 2.1. Chemicals

Ferulic acid, umbelliferone, apigenin, and  $\beta$ -CD were purchased from Sigma-Aldrich (St. Louis, MI, USA) and used without further purification. Boropinic acid, GOFA, 7-isopentenyloxycoumarin, auraptene, and umbelliprenin have been synthesized in our laboratories as previously reported, their purity (>98.8%) assessed by

Table 1	
Chromatographic gradient program.	

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Time (min)	Flow (mL/min <sup>-1</sup> )	%A <sup>a</sup>	%B <sup>b</sup>	
0,01	1,20	60,0	40,0	
18,00		60,0	40,0	
22,00		20,0	80,0	
25,00		20,0	80,0	
29,00		60,00	40,00	
33,00		60,00	40,00	
37,00		0,00	100,0	
38,00		0,00	100,0	
39,00		60,00	40,00	
50,00		60,0	40,0	

<sup>a</sup> HPLC grade water + 0,3% acetic acid (v/v).

<sup>b</sup> Acetonitrile + 0,3% acetic acid (v/v).

HPLC and <sup>1</sup>H NMR [13], and used as pure chemical standards for HPLC analysis. Methanol (HPLC grade), acetonitrile (HPLC grade), and acetic acid were purchased from Carlo Erba Reagents (Milan, Italy). HPLC-grade water (>  $18 M\Omega/cm$  resistivity) was obtained by passage through an Elix 3 and Milli-Q academic water purification system (Millipore, Bedford, MA, USA).

#### 2.2. Extraction procedures

Roots, stems, leaves, and flowers of A. retroflexus, have been collected in May 2016 in Montelongo (Campobasso, Molise region, Italy) (GPS coordinates 41° 44′ 08″ N, 14° 56′ 49″ E). The plant was identified by Francesco Epifano and Salvatore Genovese. Voucher specimens (AR-R-2016-1, AR-S-2016-2, AR-L-2016-3, and AR-F-2016-4) have been stored in the deposit of the laboratory of Chemistry of Natural Compounds at the Department of Pharmacy of the University "G. D'Annunzio" of Chieti-Pescara. All samples have been dried, ground, and homogenized prior to extraction experiments. 1g of each plant samples was extracted with 10 mL of each of the following solvents: *n*-hexane, petroleum ether, CH<sub>2</sub>Cl<sub>2</sub>, diethyl ether, ethyl acetate, acetone, acetonitrile, 2-propanol, EtOH, H<sub>2</sub>O/EtOH 7:3, MeOH, and H<sub>2</sub>O containing 1.5% β-CD. Extractions were accomplished using three different methodologies: 96 h maceration, ultrasound- (room temperature for 10 min) and microwave- (100 W, equivalent to a temperature of 50 °C) assisted extractions. When used,  $\beta$ -CD has been precipitated by centrifugation (13000 rpm) and filtered. The solid formed has been then washed with MeOH (1 mL) and the filtrate evaporated to dryness. The powder obtained was re-suspended in of MeOH (100  $\mu$ L) and an aliquot of 20 µL of this solution injected into the HPLC apparatus.

## 2.3. HPLC

Analysis were performed using a Waters 600 HPLC system equipped with a Waters 2996 PDA detector, a Rheodyne manual syringe-loading valve injector model 7125 (Cotati, CA., USA) fitted with a 20  $\mu$ L loop. Data acquisition was monitored by Waters Empower software (ver. 2.0). Chromatographic separation was achieved employing a GraceSmart RP<sub>18</sub> (5  $\mu$ m particle size, 250 mm × 4.6 mm, Grace, Deerfield, IL, USA). Column temperature was maintained at 25 ± 1 °C using a cool pocket chiller (Thermo-Scientific, Waltham, USA). The detection was set at 322 nm for each analyte. Elution mixture consisted of H<sub>2</sub>O and acetonitrile both with 0.03% of acetic acid (eluent A and eluent B respectively). Mobile phase was directly on-line degassed by using Infinity Agilent model 1260 (Agilent Technologies, Santa Clara, CA, USA). The flow rate was 1.20 mL/min. Chromatographic separation was carried out using the gradient elution reported in Table 1. Download English Version:

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