PHYTOCHEMICAL STUDY AND ANTIRADICAL ACTIVITY

OF Artemisia coerulescens FROM ABRUZZO

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Artemisia coerulescens L. (Asteraceae), also known by several common names such "Assenzio marittimo" or "Santonico", is a perennial herb widespread along the maritime coasts of the Mediterranean region. Aerial parts of A. coerulescens, especially flowers, are used for culinary purposes and as a stomachic, digestive, antipyretic, and antihelmintic agent [1]. Many subspecies and chemotypes of this plant have been described. The chemical composition of the essential oil of A. coerulescens collected from many parts of Europe has been investigated [2-3] and some differences between plants collected in different areals have been detected.

In the present communication we report results obtained on the qualitative and quantitative analysis of some secondary metabolites extracted from *A. coerulescens* grown in Abruzzo (Central Italy).

The plant (aerial parts) was collected in July 2005 around Atri (Teramo, Abruzzo, Italy) and air dried. A voucher specimen (No. AC-01-2005) has been deposited at the Dipartimento di Scienze del Farmaco, University "G. D'Annunzio" of Chieti-Pescara, Italy.

The essential oil of aerial parts of *A. coerulescens* was obtained by steam distillation using a Clevenger apparatus. GC and GC/MS analysis were carried out as already described [4], and the chemical composition of the essential oil is reported in Table 1.

The major components have been found in the monoterpene ketone fraction and were α -thujone (60.64%), β -thujone (23.04%), and camphor (13.84%), the total amount of which represents more than 97% of whole volatile compounds found in the essential oil of *A. coerulescens*. These data represent, compared to already reported ones for the same species [2, 3, 5], a peculiar feature of *A. coerulescens* from aerals of Abruzzo, so that it may be claimed as a novel chemotype extremely rich in monoterpene ketones.

Analysis of flavonoids and other phenylpropanoids contained in the hydroalcoholic extract of aerial parts from *A. coerulescens* were carried out as already reported [6], and the results are expressed as mg/g of dry vegetable material and are reported in Table 2.

Seven flavonoids and two cinnamic acid derivatives were identified: chlorogenic acid, caffeic acid, vitexin, hyperoside, apigenin-7-*O*-glucoside, myricetin, luteolin, kaempferol, and quercetin. As reported in Table 2, caffeic acid, luteolin, kaempferol, apigenin-7-*O*-glucoside, and quercetin were the main components.

Finally we analyzed the chemical composition of the extract obtained by a 48 hours maceration of the aerial parts (10 g) with dichloromethane (300 mL). After purification by SiO_2 gel column chromatography by elution with CH_2Cl_2 , we isolated two main fractions: the less polar one was a mixture of fatty acids whose composition, analyzed by GC-MS after derivatization as methyl esters by reaction with an ethereal solutuion of diazomethane, was the following: palmitic acid (34.85%), myristic acid (28.15%), stearic acid (35.26%), oleic acid (1.26%), and linoleic acid (0.48%). The second fraction contained a single pure compound, the structure of which was determined by 1H NMR, ^{13}C NMR, JMODXH, IR spectroscopies, and GC/MS spectrometry. The recorded data were in full agreement with those reported in the literature for desoxy- ψ -santonin [7].

This sesquiterpene lactone has been previously isolated from some plants belonging to the genera Artemisia, Dioscorea, and Tanacetum [8-10], but it is the first time that desoxy- ψ -santonin has been isolated from A. coerulescens.

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TABLE 1. Chemical Composition of the Oil of Aerial Parts of A. coerulescens

Compound	% ^a	RI
lpha-Thujene	0.33	931
Camphene	0.28	952
1,8-Cineol	0.20	1025
α -Thujone	60.64	1100
β -Thujone	23.04	1113
β -Terpineol	0.63	1137
Camphor	13.84	1139
Borneol	0.85	1164
Menthol	0.19	1172

^aData are mean of three measurements.

TABLE 2. Flavonoids and Cinnamic Acid Derivative Composition of Hydroalcoholic Extract of Aerial Parts of *A. coerulescens*

Compound	mg/g dry material ^a
Chlorogenic acid	2.2
Caffeic acid	43.0
Vitexin	2.0
Hyperoside	8.6
Apigenin-7- <i>O</i> -glucoside	80.0
Myricetin	0.9
Luteolin	30.0
Kaempferol	70.0
Quercetin	120.0

^aData are mean of three measurements.

Although described as the main components for *A. coerulescens* subsp. gallica collected in Spain [11], α - and β -santonin were not detected for the plant grown in Abruzzo.

Scavenging free radical potentials were tested in a methanolic solution of DPPH [12]. The degree of decoloration of the solution indicates the scavenging efficiency of the added sample. For the methanolic extract, $100 \,\mu\text{L}$ was added to $0.9 \,\text{mL}$ of pure methanol and $4 \,\text{mL}$ of DPPH solution (final concentration of DPPH: $2.0 \times 10^{-4} \,\text{M}$). Thirty minutes later, the absorbance was measured at 517 nm. A blank solution was prepared with $100 \,\mu\text{L}$ of methanol. The scavenging activity on DPPH radical was expressed as EC_{50} , which is the concentration of the test extract required to give a 50% decrease of the absorbance from that of the blank solution.

The methanolic extract was also shown to scavenge directly the stable DPPH radical with an EC_{50} value of 697 μ g + 31 μ g. The value shows that the methanol extract does not have strong antiradical activity.

In conclusion, in this study we reported a re-investigation of the phytochemical composition of A. coerulescens grown in Abruzzo (Central Italy). We have described a secondary metabolite, desoxy- ψ -santonin, described in this species for the first time, and we pointed out some main differences in the composition of the essential oil of the title plant compared to the essential oils of plants collected in other parts of Europe. Results reported herein suggest also that A. coerulescens grown in Abruzzo may be claimed as a novel chemotype especially when referring to the monoterpene ketone composition and lack of sesquiterpene lactone typical of the genus Artemisia, such as α - and β -santonin.

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