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Short Communication

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Total Phenolic Content and Antioxidant Activity of Extracts of *Achyrocline satureioides* Flowers from Different Zones in Argentina

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SUMMARY. ***Achyrocline satureioides*** **is widely used, along Argentina, Brazil and Uruguay, in popular medicine. In this study we compared the total phenolic content, expressed as gallic acid equivalent/g (mg of GAE/g of sample), using methanolic extracts of *A. satureioides* flowers collected in different zones of Argentina. The extracts were also tested for their antioxidant activity monitoring the bleaching rate of 1,1-diphenyl-2-picrylhydrazyl (DDPH). The polyphenol content, as well as the scavenging activity showed a great variability (23.0-112.6 mg GAE/g and DPPH IC_{50} 4.47-23.98 μg/ml) depending on the origin of the samples. Interestingly, the phenolic content correlates with scavenging activity, but the results also indicate that cultivations of this medicinal plant should be necessary in order to assure uniformity and quality.**

RESUMEN. "Contenido en Fenoles Totales y Actividad Antioxidante de Extractos de Flores de *Achyrocline satureioides* provenientes de Diferentes Zonas de Argentina". *Achyrocline satureioides* es ampliamente empleada, a lo largo de Argentina, Brasil y Uruguay, en medicina popular. En este estudio, hemos comparado el contenido de fenoles totales, expresados como equivalente/g de ácido gálico (mg de GAE/g de muestra), utilizando extractos de flores de *A. satureioides,* colectadas en diferentes zonas de Argentina. Los extractos fueron además evaluados por su actividad antioxidante, monitoreando la velocidad de decoloración del 1,1-difenil-2-picrilhidrazil (DDPH). El contenido de polifenoles, así como la actividad eliminadora de radicales libres, mostró una gran variabilidad (23,0-112,6 mg GAE/g y DPPH IC_{50} 4,47-23,98 μg/ml) dependiendo del origen de las muestras. Es interesante destacar que el contenido de fenoles se correlaciona con la actividad eliminadora de radicales libres, aunque los resultados indican además la necesidad de obtener cultivos de esta planta medicinal, con el propósito de asegurar su uniformidad y calidad.

INTRODUCTION

Achyrocline satureioides (Lam.) DC (Asteraceae), commonly known as "marcela" or "macela", is a medium-sized aromatic annual herb indigenous of subtropical South America. Due to its widely dispersion, it is extensively used in popular medicine for a variety of ailments. Although this species is largely used in popular medicine, it is not generally cultivated in Argentina. Moreover, the commercial plants are harvested in their natural habitat which posses ecological and edaphic differences.

The infusions of the aerial parts and/or flowers are frequently used in folk medicine and as local phytotherapic agents and are present in bitter and digestive beverages. Some not demonstrated uses (sedative, antiepileptic, antitumoral, emmenagogue, vermifugue) have been reported [1]. Besides, other uses have been scientifically demonstrated: hepatoprotective [2], vasodilatory [3], as well as antiulcerative, antispasmodic, antiviral and anti-inflammatory [4] activities, representing this last property the most relevant traditional use. Inflammation was linked to several physiological problems, being free radicals one of the main disruptors of these damages. For this reason antioxidant activity should be well established, and correlated with the most feasible active constituents: polyphenols. Flavonoids [5], caffeic and chlorogenic acids

KEY WORDS: *Achyrocline satureiodes,* Free radical, Phenolic content.
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and esthers [6], coumarins [7] and essential oil [8] have been reported to be present in *A. satureioides*.

In this investigation, eight samples of *A. satureioides* ("marcela") were collected during summer 2007 in different zones or districts of Argentina, representing the most important areas of distribution of this species in our country.

MATERIALS AND METHODS

Voucher specimens (1/1894, 2/1889, 3/1895, 4/1611, 5/1554, 6/1562, commercial sample 7 and 8/1908) were identified by Drs. M. and S. Gattuso from the Department of Botany, University of Rosario and stored in the Chair of Pharmacognosy, University of Buenos Aires, Argentina. Detailed sample information is included in Table 1.

Dry flowers (0.2 g) of the different samples were extracted with 5 ml of 70% methanol during 10 min at 70 °C. The extracts were stored at room temperature for 6 h and then centrifuged at 5000 rpm for 10 min, twice. The supernatants liquids were collected and combined and then completed to final volume of 10 ml with 70% methanol.

For the determination of the antioxidant activity the extraction procedure was the same as for the determination of the total phenolic compounds, but after completing to final volume, the extract was taken to dryness *in vacuo* at 40 °C. 5 mg of each dry extract were dissolved in 5 ml methanol for the free radical scavenging activity determination.

Total phenolic content

The concentration of total phenolic compounds of the extracts was determined by the Folin-Ciocalteu method [9] using the extracts at a dilution of 1:100 in water. The absorbance of the samples was measured at 765 nm. The results are expressed as mg of gallic acid equivalent (GAE)/g of each sample.

Antioxidant activity

Scavenging activities of the extracts on the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were assayed using a modified Blois method [10], by which the bleaching rate of DPPH is monitored at a characteristic wavelength in presence of the sample. A volume of 0,1 ml of different concentrations of extracts from 1000 to 1 µg/ml, with 0,5 ml of a 500 µM DPPH solution in ethanol and 0,4 ml of 0.1 M buffer of Tris-HCl, at pH 7,4. The absorbance was measured at 517 nm after 20 min of reaction in the darkness. Ascorbic acid solutions of different concentrations were used as positive controls for antioxidant activity. The percentage decrease of DPPH was calculated applying the following equation: % of inhibition= [1- (As / A0)] x 100, where As is the absorbance of the sample, A0 = is the absorbance of the DPPH solution.

The IC_{50} values were calculated from data obtained graphically, using the principle of a right-angled triangle: CE_{50}= D- [(A-50 % max response). X]/ Y, in which A is the immediately higher response of 50% max response; B is the immediately lower response of 50% max response; D = log concentration corresponding to A response; C = log concentration corresponding to B response; X = D-C; Y = A-B [11]. The results were expressed as mean ± SEM of three determinations made by duplicate.

Statistical analysis

One way analysis of variance was performed by ANOVA procedures. Significant differences between IC_{50} of different extracts were determined by the Newman -Keuls test. $P< 0.05$ was considered as significant.

Sample/ Voucher Nº	Recollection Place	Total phenolics (mg of GAE /g of sample)	DPPH IC50 µg/ml
1 /1894	Ptdo. Cnel. Rosales, Province of Buenos Aires	112.6	4.47 ± 0.21
2 /1889	Ptdo. Tornquist, Province of Buenos Aires	104.5	7.80 ± 0.50
3/1895	Ptdo. Cnel. Rosales, Province of Buenos Aires	97.0	9.00 ± 0.50
4/1611	Agua de Oro,Province of Córdoba	59.6	10.08 ± 1.00
5 /1554	Capilla del Monte, Province of Córdoba	45.2	12.00 ± 1.00
6 /1562	Los Reartes, Province of Córdoba	37.5	13.48 ± 1.30
7	Commercial sample	32.7	13.18 ± 1.00
8 /1908	La Florida, Province of San Luis	23.0	23.98 ± 1.50

Table 1. Polyphenol contents (mg of GAE/g of sample) and IC_{50} values against DPPH (µg/ml) in methanolic extracts of *A. satureioides* flowers.

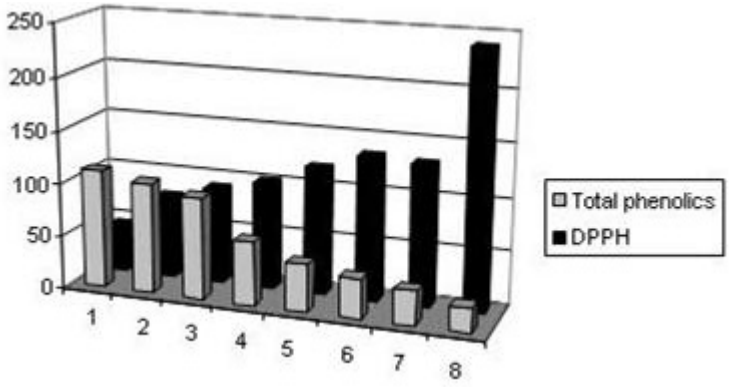

Figure 1. Polyphenol contents (mg of GAE/g of sample) and IC_{50} values against DPPH (μg/ml) corresponding to Table 1.

RESULTS AND DISCUSSION

The results are given in Table 1 and Figure 1.

The comparison of polyphenol contents and antioxidant activity of "marcela" flowers, collected in several zones representing mostly the distribution area of the species in Argentina was the aim of this study, in order to evaluate the homogeneity of the natural locations.

The total polyphenol content in the analyzed methanolic extracts varied from 23.0 to 112.6 mg of GAE/g of each sample. It is well known that the qualitative and quantitative composition of phenolic compounds depends on numerous factors, particularly plant chemotype and growth conditions (rain, soil, temperature, etc.). The progressive increase of total polyphenolic compounds found discards the probable presence of chemotypes. However the influence of ecological factors may explain the wide variation of concentrations detected in the methanolic extracts, suggesting the possible presence of ecotypes or an expression of the plasticity of this species.

The same extracts were all positive for free radical scavenging activity, but showed again a great variability (IC_{50} from 4.47 to 23.98 μg/ml). Interestingly, the results obtained show a good correlation between the polyphenols content and the antioxidant activity.

The correlation between polyphenol content and antioxidant activity could support the popular use in inflammatory diseases which are extensively related to tissue damage induced by free radicals.

Our findings confirm that this species should be cultivated for warranting quality and uniformity if it is used for the radical scavenging activity in phytotherapy, dietary supplements or cosmetics but previously multidisciplinary agronomical studies should be carry on in order to associate different ecological and subsequent crops patterns with polyphenolic contents.

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