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Antioxidant potential of *Baccharis incarum*: isolation of bioactive metabolites

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INTRODUCTION

The genus *Baccharis,* one of the important genera of the tribe Astereae includes approximately 500 species. Its geographical distribution is exclusively American. It is represented by 96 species in Argentina (Giuliano, 2001). The selected species for the present study, *Baccharis incarum*, grows at 3800 meters above sea level (Antofagasta de la Sierra, Province of Catamarca, Puna de Atacama, Argentina) in areas of arid climate, low atmospheric pressure, wide range of temperatures and high solar radiation. It is popularly known as 'bleach' ('lejía') and it is commonly used by the inhabitants to protect stomach and liver, restore blood circulation, reduce inflammatory processes and cure ulcers, burns and skin wounds. It is also used as fuel and cattle fodder. The aim of the present work was to evaluate the antioxidant potential of aerial parts of *B. incarum* and to isolate and purify the compound responsible for the activity.

METHODOLOGY

Plant material was collected in February 2005 in Antofagasta de la Sierra, Catamarca, Argentina. Tinctures in 80% ethanol were prepared, and the content of total phenolic compounds (Singleton *et al.*, 1999), flavones, flavonols, flavanones and dihydroflavonones (Popova *et al.*, 2005) was determined.

Antioxidant activity evaluation. DPPH free radical scavenging capacity (Yamaguchi *et al.*, 1998), ABTS cation radical (Re *et al.*, 1999), superoxide (Xanthine/xanthine oxidase/tetrazolium blue/EDTA) (Kong *et al.*, 2000), hydroxyl (deoxyribose/$FeCl_3$/EDTA/ascorbic acid/H_2O_2) and hydrogen peroxide (Aruoma, 1987) were determined. The reducing power was evaluated (potassium ferricyanide/trichloroacetic acid/$FeCl_3$) (Yen *et al.*, 1993), and its activity was compared with that of synthetic and natural antioxidants.

The phytochemical profile of the species was analyzed.

Extraction and isolation of bioactive compounds. Desiccated material of *B. incarum* was successively extracted with petroleum ether, ethyl ether and methanol. The extract obtained with ethyl ether was evaporated to dryness and further suspended in ethanol. It was subjected to a chromatography column using silica gel G60 and eluted stepwise with chloroform and then with mixtures of chloroform-methanol in increasingly polar ratios.

The collected fractions were analyzed by TLC (solvent system: chloroform: ethyl acetate 10%) and assayed for antioxidant activity by autography. The active fractions were subjected to a purification process by HPLC. The identification of the bioactive metabolites was carried out by UV-visible spectra and thin-layer chromatography. Structural elucidation will be carried out by Nuclear Magnetic Resonance spectroscopy (^{1}H-NMR).

RESULTS AND DISCUSSION

The *B. incarum* ethanolic extract showed a high flavonoid content, mainly flavones. It showed capacity to donate electrons in the presence of the DPPH free radical (IC_{50} values of 25 microg/mL) and TEAC values of 7.833/100 g dry weight. In the non-enzymatic system of O_2^- generation, the extract turned out to be a scavenger of superoxide radicals. It was also effective scavenging hydroxyl radicals and H_2O_2.

The bioguided isolation of antioxidant metabolites with antioxidant capacity was carried out by successive extractions with different solvents, preparative column chromatography and high resolution chromatography. Two main active compounds, C1 and C2 (Fig. 1), were obtained. According to spectral analysis and thin-layer chromatography developed with different chemical systems, the bioactive compounds of *Baccharis*

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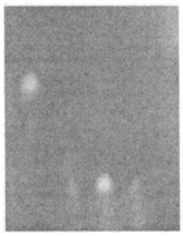
accounted for methoxylated flavones.



C1 C2

Figure 1. Bioguided isolation of metabolites with antioxidant activity: Autography using $ABTS^{\cdot+}$ immobilized in gel.

CONCLUSIONS

We have previously demonstrated that the ethanolic extracts of *B. incarum* show a high antimicrobial capacity. This finding together with the antioxidant and scavenging capacity of reactive oxygen species demonstrated in this work would indicate the potential applications in the pharmaceutical and food industry of this specie. On the other hand, although we isolated two of the metabolites responsible for the antiradical activity, further studies are necessary to confirm the structural identity.

Note: This study was presented at the 'I Reunión de Biotecnología aplicada a plantas medicinales y aromáticas' (First Biotechnology Meeting on Medicinal and Aromatic Plants), Córdoba, Argentina, 2006.

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