

## Antioxidant activity of *Baccharis spicata*, *Baccharis trimera* and *Baccharis usterii*

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**SUMMARY.** Antioxidant activities of extracts and fractions from *Baccharis spicata*, *B. trimera* and *B. usterii* were determined using TRAP and TBARS assays. Aqueous extracts from *B. spicata* and *B. trimera*, at a concentration of 25 µg/mL, showed a higher antioxidant activity when compared to Trolox® (1 mM) and aqueous extract from *B. usterii* exhibit similar activity than Trolox®. Fractions were tested using 2.5 µg/mL. In TBARS all extracts and fractions were effective in the prevention of lipid peroxidation by inhibiting the formation of thiobarbituric acid reactivities species and cell mortality induced by hydrogen peroxide.

**RESUMEN.** "Actividad antioxidante de *Baccharis spicata*, *Baccharis trimera* y *Baccharis usterii*". Para determinar la actividad antioxidante de los extractos y de las fracciones de *Baccharis spicata*, *B. trimera* y *B. usterii* se emplearon los ensayos TRAP e TBARS. Los extractos acuosos de *B. spicata* e *B. trimera*, a una concentración de 25 µg/mL, presentaron actividad antioxidante mayor que Trolox® (1 mM) y para el extracto acuoso de *B. usterii* se observó una actividad semejante al Trolox®. Las fracciones fueron ensayadas a una concentración de 2,5 µg/mL. En TBARS todos los extractos fueron efectivos en la prevención de la peroxidación lipídica a través de la inhibición de la producción de especies reactivas del ácido tiobarbitúrico y de la mortalidad de las células por el peróxido de hidrógeno.

### INTRODUCTION

*Baccharis spicata*, *Baccharis trimera* and *Baccharis usterii*, members of Asteraceae, are native species to South Brazil, Paraguay, Uruguay and Argentina. Their aerial parts have been used in folk medicine as diuretic and digestive <sup>1</sup>. Previous studies reported the identification of flavonoids, diterpenes and saponins from *B. trimera* demonstrating also antihepatotoxic, anti-inflammatory, analgesic, antimutagenic activities and vascular relaxant effect <sup>2-7</sup>. So far, no chemical or biological investigations have been carried out on *B. spicata* and *B. usterii*.

There is an increasing interest in the antioxidant effects of compounds derived from herbs that could be relevant in relation to their nutritional incidence effects and their role in health and disease <sup>8</sup>. Several diseases of the gastrointestinal tract seem to be induced by oxidative

stress. The role of oxygen-derived free radicals has been studied in acute gastric and esophageal mucosal injury caused by ischemia, anti-inflammatory drugs, or ethanol. Administration of free radical scavengers has been found to prevent esophageal mucosal damages <sup>9</sup>. These suggest that the folk use of *B. spicata*, *B. trimera* and *B. usterii* could be associated with antioxidant properties.

Recently, we could demonstrate that compound BaiI (4'-O-β-D-glucopyranosyl-3',5'-dimethoxybenzyl-caffeoate) isolated from the *n*-butanol fraction of *B. articulata* aerial parts has antioxidant activity comparable to Trolox® <sup>10</sup>.

This paper reports the antioxidant activity of crude dichloromethane, ethanol and aqueous extracts from *B. spicata*, *B. trimera* and *B. usterii* aerial parts through the evaluation of total antioxidant potential (TRAP), the prevention of

**KEY WORDS:** Antioxidant activity, *Baccharis*, Free radicals, Lipid peroxidation.

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formation of thiobarbituric acid reactive species (TBARS) induced by hydrogen peroxide and by determination of the protection of Sertoli cells against hydrogen peroxide induces cell damage. Fractions from the aqueous extract (dichloromethane, ethyl acetate, *n*-butanol and aqueous residue) were also evaluated.

## MATERIAL AND METHODS

### Plant material

Aerial parts of *B. spicata* (Lam.) Baillon, *B. trimera* (Less.) DC. and *B. usterii* Heering were collected in Porto Alegre, State of Rio Grande do Sul, Brazil, in February 2001. Herbarium specimens are on deposit in the Herbarium of the Botany Department of Universidade Federal do Rio Grande do Sul, Porto Alegre. Plant materials were air-dried and powdered.

### Preparation of plant extracts and fractions

Evaluation of the antioxidant activity was performed first on the dichloromethane, ethanol and water crude extracts. The dichloromethane extract was obtained by refluxing the plant (1 g, 2 x 100 mL) during 4 h. The ethanol extract was prepared by maceration (1 g, plant:solvent, 1:10, w/v) (2 x 10 days). The aqueous extract was obtained by decoction of the plant material (1 g, 2 x 100 mL) during 30 min. The yields of the extracts were (mg): for *B. spicata* 85 ( $\text{CH}_2\text{Cl}_2$ ), 210 (EtOH), 230 ( $\text{H}_2\text{O}$ ); for *B. trimera* 80 ( $\text{CH}_2\text{Cl}_2$ ), 425 (EtOH), 290 ( $\text{H}_2\text{O}$ ); for *B. usterii* 70 ( $\text{CH}_2\text{Cl}_2$ ), 530 (EtOH), 270 ( $\text{H}_2\text{O}$ ).

After preliminary assays, the aqueous extracts of *B. spicata* (230 mg), *B. trimera* (290 mg) and *B. usterii* (270 mg) were partitioned, separately, using dichloromethane, ethyl acetate and *n*-butanol yielding the respective fractions together with an aqueous residue. The yields were (mg): for *B. spicata* 7 ( $\text{CH}_2\text{Cl}_2$ ), 2 (AcOEt), 104 (BuOH), 100 (aqueous residue); for *B. trimera* 14 ( $\text{CH}_2\text{Cl}_2$ ), 48 (AcOEt), 200 (BuOH), 26 (aqueous residue); for *B. usterii* 4 ( $\text{CH}_2\text{Cl}_2$ ), 48 (AcOEt), 90 (BuOH), 125 (aqueous residue). Each extract or fraction was evaporated to dryness under reduced pressure.

### Reagents and Instrumentation

Thiobarbituric acid, hydrogen peroxide, luminol were purchased from Sigma, St. Louis, MO. 2,2'-azobis-(2-methylpropionamidine) dihydrochloride (AAPH) and Trolox® were purchased from Aldrich Chemical Co., Milwaukee, WI. Scintillation counting was performed on a Beckman instrument.

### Antioxidant activity

The *in vitro* antioxidant potential of *B. spicata*, *B. trimera* and *B. usterii* extracts and fractions were estimated by the total radical-trapping antioxidant parameter (TRAP). The principle of TRAP measurement has been described previously <sup>9</sup>. Briefly, the reaction was initiated by injecting luminol and AAPH in glycine buffer that resulted in steady luminescence emission. The addition of different concentrations of plant extracts/fractions and Trolox® (1 mM, as the standard antioxidant) decreases the luminescence proportional to its antioxidant potential. The luminescence emission was followed by 70 minutes after the addition of plant extracts or Trolox®. The decrease in the luminescence is proportional to the antioxidant potential. Results are expressed as percentage of control, which receive only the extracts solvent (extract solvent = 100%). Values are expressed as means ± S.D. (n= 4 each group) <sup>11</sup>.

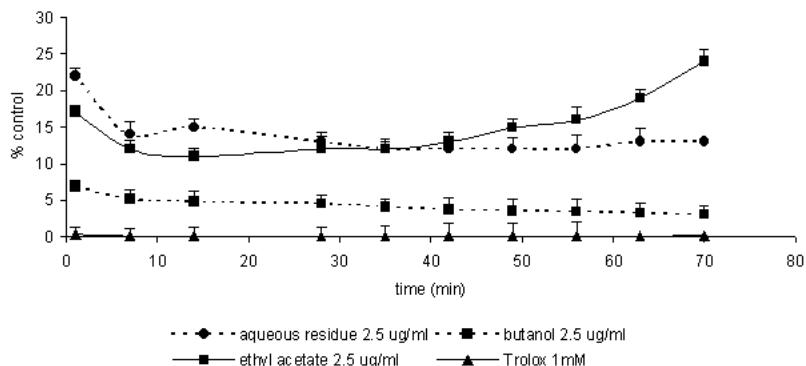
The *ex vivo* antioxidant potential of *B. spicata*, *B. trimera* and *B. usterii* was estimated by the prevention of formation of thiobarbituric acid reactive species (TBARS), and by the determination of cell viability in cultured rat Sertoli cells exposed to hydrogen peroxide as previously described <sup>12</sup>. Cells were treated with crude ethanol and aqueous extracts (in concentrations of 2.5 and 25 µg/mL), *n*-butanol and ethyl acetate fractions from aqueous extracts and the aqueous residues (in concentrations of 2.5 µg/mL) for 24 h. Control cells received the same volume of solvent. TBARS production was estimated by absorbance at 532 nm, and expressed as malondialdehyde (MDA) equivalents (nm/mg protein). Cell viability was determined by the Trypan blue exclusion test <sup>13</sup>.

Results are expressed as means and p values were considered significant when p < 0.05. Differences in experimental groups were determined by ANOVA. Comparison between means was carried out using a Newman-Keuls test.

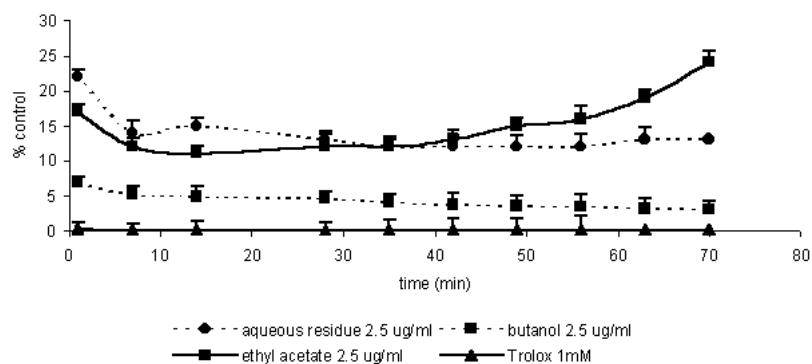
## RESULTS AND DISCUSSION

Extracts from *B. spicata*, *B. trimera* and *B. usterii* were assayed to the activity as inhibitors of lipid peroxidation and scavengers of free radicals. The total antioxidant potential (TRAP), the thiobarbituric acid reactive species (TBARS) and the cell viability were evaluated in different extracts and fractions from aerial parts of these species.

Evaluation of TRAP levels of crude ethanol and aqueous extracts from three species exhibit-



**Figure 1.** TRAP of fractions from *B. spicata* aqueous extract. Fractions (2.5 µg/mL) from aqueous extract of *B. spicata* (ethyl acetate, butanol and aqueous residue) were evaluated as described under "Material and Methods".



**Figure 2.** TRAP of fractions from *B. trimera* aqueous extract. Fractions (2.5 µg/mL) from aqueous extract of *B. trimera* (ethyl acetate, butanol and aqueous residue) were evaluated as described under "Material and Methods".

ed a dose-dependent antioxidant activity in concentrations of 2.5 and 25 µg/mL. All crude dichloromethane extracts did not exhibit significant antioxidant activity (data not shown). Aqueous extracts of *B. spicata* and *B. trimera* at a concentration of 25 µg/mL, showed a higher antioxidant activity when compared to ethanol extracts and both extracts presented higher antioxidant activity than that of Trolox® (1 mM) (data not shown). Ethanol and aqueous extracts from *B. uesterii*, at a concentration of 25 µg/mL, showed an antioxidant activity similar to Trolox® (1 mM) (data not shown).

Dichloromethane, ethyl acetate, *n*-butanol fractions and aqueous residues were tested in concentrations of 2.5 µg/mL to evaluate TRAP levels. *n*-Butanol fractions from *B. spicata* and *B. trimera* showed a higher antioxidant activity when compared to aqueous residues and ethyl acetate fractions (Figs. 1 and 2). The *n*-butanol fraction and aqueous residue of *B. uesterii* exhibited a similar antioxidant activity that was lesser than that of Trolox® (1 mM) while the ethyl acetate fraction did not exhibited antioxidant activity (data not shown). All these fractions exhibited less antioxidant activity than that of Trolox® (1 mM). Dichloromethane fractions from all tested species did not exhibit significant antioxidant activity (data not shown).

The antioxidant activity of the crude extracts (ethanol and aqueous) and fractions (ethyl acetate, *n*-butanol and aqueous residue) from all three species was confirmed by measuring the inhibition in the production of malondialdehyde (MDA) and related substances that react with thiobarbituric acid (TBARS). All samples tested inhibited lipid peroxidation causing a significant decrease in MDA production in TBA-reactant assay. Also, these extracts and fractions presented capacity of inhibiting cell mortality induced by hydrogen peroxide (data not shown). None of these extracts induced a decreased in cell viability assessed by trypan blue test (data not shown).

The results showed that among the partitions, the more polar ones are those having higher antioxidant activity. The most active fractions in the case of *B. spicata* and *B. trimera* were the *n*-butanol, in the case of *B. uesterii* both the aqueous and the *n*-butanol fractions showed similar antioxidant activity. Recently, we observed that the *n*-butanol fraction was the most active in the case of *B. articulata* and from this fraction we could isolate a new compound with high antioxidant activity, elucidated as 4'-O-β-D-glucopyranosyl-3',5'-dimethoxybenzyl-caffeoate, named BallI<sup>10</sup>. The chromatographic comparison among aqueous extracts and *n*-butanol fractions from herein investigated *Baccha-*

*Baccharis* species indicated different chemical compositions; moreover, BaII could not be detected in these samples. Results obtained by our group through TLC techniques indicate the presence of phenolic derivatives and terpenoids in all extracts.

Flavonoids, coumarins, phenylpropanoids and terpenoids of plant origin have been reported as scavengers and inhibitors of lipid peroxidation<sup>14</sup>. Literature data report the presence of diterpenoids, flavonoids and saponins for *B. trimera*<sup>2-5</sup> that could be related to its potential antioxidant activity.

Nowadays, *Baccharis* species continue to

play an important role in the traditional medicine of many modern cultures, being used for the treatment of gastrointestinal disorders, heart failure, and hepatic alterations. Further investigations intended to confirm the antioxidant activities reported herein, as well as the isolation of the active principles, are being conducted.

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