Antisecretory actions of Baccharis trimera (Less.) DC aqueous extract and isolated compounds: Analysis of underlying mechanisms

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Aims of the study: To investigate the mechanisms involved in the antacid action of AE and isolated compounds from Baccharis trimera.

Materials and methods: AE was assayed in vivo in cold-restraint stress gastric ulcers and in pylorus-ligated mice. Nine fractions (F2–F10) previously isolated from AE were assayed in vitro on acid secretion measured as [14C]-aminopyrine ([14C]-AP) accumulation in rabbit gastric glands, and on gastric microsomal H+,-K+-ATPase preparations. Chlorogenic acids (F2, F3, F6, F7), flavonoids (F9), an ent- clerodane diterpene (F8) and a dilactonic neo-clerodane diterpene (F10) have been identified in these fractions.

Results: Intra-duodenal injection of AE (1.0 and 2.0 g/kg) in 4 h pylorus-ligated mice decreased the volume (20 and 50%) and total acidity (34 and 50%) of acid secretion compared to control values. Administered orally at the same doses AE protected against gastric mucosal lesions induced in mice by restraint at 4°C. Exposure of isolated rabbit gastric glands to fractions F8 (10–100 μM) and F9 (10–300 μg/ml) decreased the basal [14C]-AP uptake by 50 and 60% of control (Ratio = 6.2 ± 1.1), whereas the remaining fractions were inactive. In the presence of the secretagogues F2 and F4 (30–300 μg/ml) decreased the [14C]-AP uptake induced by histamine (His) with a 100-fold lower potency than that of ranitidine. F5 and F6 reduced the [14C]-AP uptake stimulated by carbachol (CCh), but they were 10 to 20-fold less potent than atropine. F8 (diterpene 2) and F9 (flavonoids) decreased both the His- and CCh-induced [14C]-AP uptake, whereas F10 (diterpene 1) was inactive against the [14C]-AP uptake stimulated by secretagogues. Diterpene 2 was the most active of all tested compounds being 7-fold less potent than ranitidine and equipotent to atropine in reducing acid secretion in vitro. This compound also reduced the gastric H+, K+-ATPase activity by 20% of control, while the remaining fractions were inactive on the proton pump in vitro.

Conclusions: The results indicate that Baccharis trimera presents constituents that inhibit gastric acid secretion by acting mainly on the cholinergic regulatory pathway. The plant extract also contains compounds that exert moderate inhibition of the histaminergic regulatory pathway of acid secretion and the gastric proton pump. Altogether these active constituents appear to provide effective inhibition of acid secretion in vivo, which may explain the reputed antiulcer activity of the plant extract.

Abbreviations: [14C]-AP, [14C]-aminopyrine; CCh, carbachol; DMSO, dimethyl sulfoxide; ECL, enterochromaffin-like cells; EDTA, ethylenediaminetetraacetic acid; H+, K+-ATPase, H+, K+-adenosine triphosphatase; His, histamine; HPLC, high performance liquid chromatography; NMR, nuclear magnetic resonance; PBS, phosphate buffered saline; TRIS, tris(hydroxymethyl)aminomethane.

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1. Introduction

A number of plant species are reputed in Brazilian folk medicine to possess antacid/antiulcer properties, one of the most popular of which is Baccharis trimera (Less.) DC (Syn. Baccharis genistelloides Persoon var. trimera (Less.) Baker; Baccharis triptera DC), family Asteraceae (Magalhães, 2000). Commonly known as “carqueja”, Baccharis trimera is one of the 120 species of the genus Baccharis found in Brazil (Verdi et al., 2005), mainly in the Southern and Eastern regions (Sousa et al., 1991). Used as infusion, decoctions or
tinctures of the aerial parts, the plant is also believed to be effective in liver diseases, diarrhea, fever, rheumatism, angina, renal disorders and diabetes (Lorenzi and Matos, 2002).

Some biological activities of Baccharis trimera have been shown experimentally. For example, antinociceptive and anti-inflammatory activities of the plant aqueous extract and its butanolic fraction have been described in rodent models (Gené et al., 1996; Paul et al., 2009). Ant hepatotoxotic activity of the plant ethyl acetate extract has been reported in a mouse model, and related to the presence of flavonoids (Soicke and Leng-Peschlow, 1987). A possible antidiabetic activity was suggested by the decrease in glycemia in mice with streptozotocin-induced diabetes after 7 days treatment with an aqueous fraction of the plant extract (Oliveira et al., 2005). Antioxidant (Simões-Pires et al., 2005), antimutagenic (Nakasugi and Komai, 1998), antimicrobial (Betoni et al., 2006), anti-hemorrhagic and anti-proteolytic activities against Bothrops snake venoms (Januário et al., 2004) have also been reported for extracts and isolated compounds from Baccharis trimera. Moreover, signs of liver and renal toxicity have been detected after 20 days of treatment in pregnant rats with the hydroethanolic extract of Baccharis trimera (Grance et al., 2008). The main constituents identified in this species are diterpenoid compounds (clerodane and labdanate types), triterpenes, flavonoids, saponins, tannins, phenolic compounds and essential oils (Sousa et al., 1991; Verdi et al., 2005).

Previous studies from this laboratory have shown that pretreatment of rats and mice with the aqueous extract of aerial parts of Baccharis trimera (AE, 5%) reduced gastric ulcers induced by 50% ethanol or cold-restraint stress, but were ineffective against those produced by nonsteroidal anti-inflammatory agents (Gamberini et al., 1991). Administered intraduodenally in pylorus-ligated rats, the AE reduced the basal gastric acid secretion as well as that stimulated by either bethanechol or pentagastrin, but not that induced by histamine (Gamberini et al., 1991; Lapa et al., 1992). This indicated the presence of active constituents in the AE that inhibit the cholinergic regulatory pathway of acid secretion.

Chemical studies have shown the presence in Baccharis trimera extract of a neo-clerodane diterpene endowed with a spasmolytic action on the vascular smooth musculature (Torres et al., 2000). Chlorogenic acids, flavonoids, and an ent-clerodane diterpene have also been identified in the plant AE (Tanae et al., 2008). This work aimed to examine the mechanisms involved in the antisecretory action of the previously isolated compounds from Baccharis trimera AE (Torres et al., 2000; Tanae et al., 2008) on in vitro rabbit gastric glands and gastric microsomal H+·K+-ATPase preparations.

2. Materials and methods
2.1. Plant material and extraction

Baccharis trimera was cultivated at the Pluridisciplinary Center of Chemical, Biological and Agronomic Studies of the University of Campinas in the state of São Paulo (Magalhães, 2000). The plant was collected in December 2000 and avoucher specimen was deposited in the herbarium of this Center as CPQBA 1286. The aerial parts of the dried plant were extracted (5%, w/v) with hot water (72 °C, 30 min), then the aqueous extract (AE) was concentrated under vacuum and freeze-dried (yield 12%, w/w).

2.2. Purification of AE

Purification of AE and chemical identification of the main active constituents were reported previously (Tanae et al., 2008). In short, AE was fractionated using a preparative HPLC system (Shimadzu, Japan) and a UV–vis detector operating at 210 nm, and a Shim-pack preparative C18 column eluted with water/acetonitrile 3–70%. Purification of AE yielded 10 fractions (F1–F10) that were analyzed on an HPLC system (Shimadzu-Japan), monitored by an SPD-M10AVP diode array detector. 1H NMR and 13C NMR spectral analysis was used for chemical identification of the main isolated constituents.

2.3. Animals

In vivo assays of acid secretion were done using F1 mice (25–30 g) (Souccar et al., 2008) that were bred in the Animal Facility at Instituto Nacional de Farmacologia e Biologia Molecular (INIFAR), Escola Paulista de Medicina/Universidade Federal de São Paulo (EPM/UNIFESP). Animals were housed under controlled temperature (22 ± 2 °C) on a 12/12 h light–dark cycle, with free access to food and water. They were deprived of solid food 15–18 h before the experiments. When necessary they were anesthetized with a mixture of xylazine (10 mg/kg) and ketamine (100 mg/kg), i.p; male white rabbits (2.5–4.0 kg) were obtained from a trusted local supplier. All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals of the USA National Institutes of Health (Bethesda, Maryland), and after approval by the local Animal Investigation Ethics Committee (License N° 761/07).

2.4. Drugs and chemicals

Drugs used were: ATP disodium salt, atropine sulfate, carbamoylcholine chloride, histamine dihydrochloride, collagenase type IA, bovine serum albumin (BSA Fraction V), dimethyl sulfoxide (DMSO), sodium thiocyanate (Sigma–Aldrich, St. Louis, MO, U.S.A.), omeprazole (Biosintética, Brazil), ranitidine (Antak®, Glaxo SmithKline, Brazil), scintillation cocktail Optiphase HISAFE II (Packard Instruments Co., CT, U.S.A.), [dimethylamine-14C]-aminopyrine (specific activity 109 mCi/mmol, GE Healthcare Life Sciences). All other chemicals were of analytical grade. For testing, the fractions were prepared in phosphate buffered saline (PBS) except fractions F8, F9 and F10 which were dissolved in DMSO (final concentration = 0.05%). Appropriate concentrations of the vehicle prepared with the incubation medium were used as control.

2.5. Experimentally induced gastric ulceration

Fasting mice were given by gavage tap water (0.1 ml/10 g body wt, p.o.), the plant extract (AE, 1.0 and 2.0 g/kg) or the H2 receptor antagonist ranitidine (0.05 g/kg, positive control). After 1 h gastric lesions were induced by stress (restraint for 2 h at 4 °C) (Senay and Levine, 1967), and at the end of this procedure animals were euthanized under anesthesia. The stomachs were dissected out, slit along the greater curvature, the mucosal side was gently cleaned and this was then examined under magnification (10 ×) by two observers unaware of the treatments. The index of mucosal damage (IMD) of each stomach was the sum of grades determined according to the following scale: loss of mucosal folds, mucosal discoloration, edema or hemorrhage (grade 1 each); less than 10 petechiae (grade 2), more than 10 petechiae (grade 3); ulcers/cm2 less than 1 mm (=number of ulcers × 2); ulcers greater than 1 mm/cm2 (=number of ulcers × 3); perforated ulcers (=number of ulcers × 4).

2.6. Pylorus ligation

Fasting mice were anesthetized and a pylorus ligation was performed as previously described (Schultz et al., 2007). The AE (1.0 and 2.0 g/kg) or the positive control ranitidine (0.05 g/kg) was then injected into the duodenal lumen (i.d.) and the abdominal wall was sutured. Control animals received corresponding volumes of saline. After 4 h the animals were euthanized under anesthesia, the gastric
secretion was collected and its final volume and pH were determined. Total acidity of the gastric juice was titrated with 0.01 N NaOH, using 2% phenolphthalein as indicator.

2.7. Gastric glands preparation

Gastric glands were prepared from rabbit gastric mucosa as described by Berglindh and Öbrink (1976). Briefly, the rabbit was deeply anesthetized with pentobarbital sodium, i.v., the stomach was perfused under pressure with phosphate buffered saline and then dissected out. The fundic mucosa was scraped off, minced and incubated in a collagenase (Sigma type IA, 0.2 mg/ml) solution for 30 min at 37 °C. The gastric glands suspension was filtered through a nylon mesh and washed several times with the incubation medium (in mM: NaCl 132.4, KCl 5.4, Na2HPO4 5.0, NaH2PO4 1.0, MgSO4 1.2, CaCl2 1.0, pH 7.4) by resuspension and gravity sedimentation.

2.8. [14C]-aminopyrine ([14C]-AP) accumulation

Accumulation of the weak base [14C]-AP was used as a measure of acid secretion by the gastric glands (Berglindh and Öbrink, 1976). Samples of the glands suspension (0.5 ml) were incubated in triplicates with either the vehicle, the test fractions or the reference drugs ranitidine (10 μM) and atropine (10 μM), the agonists histamine (1 μM) or carbachol (10 μM), and 0.05 μCi [14C]-AP (final concentration), at 37 °C in a shaker bath. After 30 min incubation, samples were centrifuged (15,000 × g, 30 s) and the supernatants were separated. The gland pellets were dried at 90 °C for 2 h, and after determination of their weights they were digested with 0.2 ml of 1 M NaOH at 90 °C, for 1 h. Samples of the supernatants and digested pellets were transferred to scintillation vials and the radioactivity was counted in a beta-counter (Packard Instruments). Non-specific trapping of [14C]-AP was determined in samples containing 10 mM NaSCN, an inhibitor of acid secretion (Hersey et al., 1981). Accumulation of [14C]-AP in the gastric glands was determined as the ratio (R) of the trapped glandular radioactivity to the radioactivity of the incubation medium (Berglindh et al., 1976).

2.9. Preparation and enzymatic assay of gastric H+, K+-ATPase

Microsomal gastric H+, K+-ATPase was prepared from rabbit gastric mucosal homogenates by Ficoll/sucrose density gradient centrifugation as described by Rabon et al. (1988). The H+, K+-ATPase enriched fraction collected at the 12% Ficoll interface was used in all assays and protein content was determined by the method of Bradford (1976). The enzymatic activity was determined in samples containing 40 mM Tris HCl buffer, pH 7.4, 0.1 mM ouabain, 20 mM KCl, 2 mM MgCl2 and 10 μg membrane protein (0.5 ml final volume), in the absence and presence of the tested fractions. The reaction was started by addition of 1.0 mM ATP. After 10 min incubation at 37 °C, the reaction was stopped by addition of 1 ml ice-cold trichloroacetic acid 10% and the released inorganic phosphate was estimated by the method of Fiske and Subbarow (1925). Samples incubated with the proton pump inhibitor omeprazole in acidified assay buffer (pH = 5.0) were used for positive control.

2.10. Statistics

Data were expressed as means ± SD. Half-maximal inhibitory concentrations (IC50) were determined from inhibition curves and expressed as geometric means and 95% confidence intervals (CI). The results were compared using one-way analysis of variance (ANOVA) followed by the Dunnett test. Non parametric data were compared using the Kruskall–Wallis test and Dunn’s post hoc test.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cold-restraint stress</th>
<th>IMD Number of ulcers cm−2</th>
</tr>
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<tbody>
<tr>
<td>Control (7)</td>
<td>26.8 ± 2.6</td>
<td>16.2 ± 2.1</td>
</tr>
<tr>
<td>AE 1.0 g/kg (6)</td>
<td>19.8 ± 3.2</td>
<td>8.8 ± 2.9</td>
</tr>
<tr>
<td>AE 2.0 g/kg (6)</td>
<td>15.7 ± 2.6</td>
<td>7.7 ± 2.9</td>
</tr>
<tr>
<td>Ranitidine 0.05 g/kg (6)</td>
<td>14.3 ± 1.7</td>
<td>5.2 ± 2.4</td>
</tr>
</tbody>
</table>

IMD, index of mucosal damage. Data are means ± SD of the number of animals in parenthesis. a p < 0.05, different from the corresponding control group (Kruskall–Wallis test and Dunn’s post hoc test). b p < 0.001, different from the corresponding control group (Kruskall–Wallis test and Dunn’s post hoc test).

All data analysis was done using the software Graphpad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Differences among data were considered significant at p < 0.05.

3. Results and discussion

Before evaluation of the mechanisms involved in the antacid and antiulcer activities of Baccharis trimera and isolated compounds, both effects of the plant AE were first confirmed in mice in vivo at doses equivalent to those used in popular medicine (Lorenzi and Matos, 2002). Pretreatment of mice with 1.0 and 2.0 g/kg of AE, p.o., protected against gastric ulcers induced by cold-restraint stress reducing the IMD by 26 and 41%, and the number of ulcers by 46 and 52% of the respective control values (Table 1). In comparison, pretreatment of mice with ranitidine (0.05 g/kg, p.o.) reduced the IMD by 47%, and the number of ulcers by 68% of control values (Table 1). In pylorus–ligated mice intraduodenal injection of 1.0 and 2.0 g/kg of AE reduced the volume by 20 and 50%, and total acidity of acid secretion by 34 and 50% of control values (1.2 ± 0.1 ml and 2.2 ± 0.2 mEq [H+] l/4h, respectively) after 4 h. The pH of the collected secretion was not significantly altered (control = 3.2 ± 0.1) with either dose (Fig. 1). These results indicate that the antiulcer activity of Baccharis trimera AE is related to inhibition of acid secretion, in agreement with previous studies (Gamberini et al., 1991; Lapa et al., 1992).

To determine the mechanisms involved in the antacid activity, nine fractions (F2–F10) obtained from the same AE (Tanea et al., 2008) were assayed on the accumulation of [14C]-AP in isolated gastric glands, a method that indirectly measures acid secretion in vitro (Berglindh and Öbrink, 1976).

In isolated rabbit gastric glands the basal acid secretion expressed as the ratio of intraglandular [14C]-AP to that present in the incubation medium was 6.2 ± 1.10 (n = 19). Incubation with histamine (His; 1, 10 and 100 μM) increased the [14C]-AP uptake in a concentration-related manner by 119 ± 7%, 193 ± 11% and 202 ± 14% of basal values, respectively. In the presence of carbachol (CCCh; 1, 10 and 100 μM) [14C]-AP accumulation in the gastric glands did not change at 1 μM, but it was increased by 168 ± 15% and 268 ± 24% of the basal value at 10 and 100 μM, respectively.

Incubation with F2–F7 (10–300 μg/ml) did not affect the basal [14C]-AP accumulation in gastric glands preparations. Previous chemical studies have identified the presence of chlorogenic acids in some fractions such as 5′-O-caffeoylquinic acid (F2), 4-O-[E]-caffeoyl-1-methyl-quinic acid (F3), 1′-5′-O-dicafeoylquinic acid (F6), and 1,3-di-[E]-caffeoylquinic acid (F7) (Tanea et al., 2008). Caffeoyl quinic acid derivatives are characteristic of Asteraceae (Clifford, 2000), and have been reported in extracts of Baccharis gaudichaudiana (Akaike et al., 2003).
Fig. 1. Effect of the aqueous extract of *Baccharis trimera* (AE, 1.0 and 2.0 g/kg) injected intraduodenally (i.d.) on the volume (A) and total acidity (B) of gastric secretion collected from mice 4 h after pylorus ligation. Control animals were given saline (C, 0.1 ml/10 g body wt, i.d.). The columns and vertical bars are means ± SE of 7 animals. ** different from control (p < 0.01).

Fractions F8 (10–100 μM), identified as an ent-clerodane diterpene (diterpene 2) ([Tanae et al., 2008](#)), and F9 (10–300 μg/ml), mainly consisting of flavonoids ([Torres et al., 2000](#); [Tanae et al., 2008](#)) decreased the basal [14C]-AP uptake at the highest concentrations (by 52% and 58% of control, respectively). Fraction F10 (10–100 μM), which contained a neo-clerodane diterpene (diterpene 1) with a smooth muscle relaxant activity ([Torres et al., 2000](#)) did not influence the basal [14C]-AP uptake in the gastric glands preparation.

Gastric acid secretion is stimulated by, (a) histamine release from enterochromaffin-like cells (ECL) in the oxyntic glands, (b) gastrin, released from G cells in the pyloric gastric glands, and (c) acetylcholine, released from postganglionic enteric neurons. Histamine-induced acid secretion is mediated by H₂ receptors on parietal cells that are coupled to the adenylate cyclase signal transduction pathway. Cholinergic and gastrin direct stimulation of acid secretion by parietal cells is mediated by M3 and CCK₂ receptors, respectively, and involves an increase in the cytosolic calcium concentration brought about via the inositol 1,4,5-triphosphate (IP₃) pathway. Acetylcholine and gastrin also stimulate acid secretion indirectly by reducing the restraint exerted by somatostatin on parietal cells and ECL, and by releasing histamine from ECL, respectively. Activation of the gastric H⁺, K⁺-ATPase (proton pump) is the common final step of acid secretion in both regulatory pathways ([Schubert, 2009](#); [Yao and Forte, 2003](#)).

Tested in the presence of the secretagogues, F2 and F4 (10–300 μg/ml) decreased the His-induced [14C]-AP-uptake by 55 and 40% of control, respectively, at the highest concentration (Fig. 2). By contrast, F3, F5, F6 and F7 (10–300 μg/ml) did not significantly change the His-induced [14C]-AP-uptake. This indicated the presence of constituents in *Baccharis trimera* AE that inhibited the histaminergic regulatory pathway of acid secretion and may contribute to the plant’s anti-ulcer activity.

In the same range of concentrations, F5 and F6 reduced the [14C]-AP accumulation induced by CCh (10 μM) by 40–45% of control (Fig. 3), whereas F2, F3, F4 and F7 were inactive. These results indicated the presence in the plant AE of constituents that also inhibited the cholinergic regulatory pathway of acid secretion. Based on the determined IC₅₀ values, when compared to the reference drugs the fractions that reduced the His-induced [14C]-AP uptake were 80 to 120-fold less potent than ranitidine, whereas those active against the CCh-induced [14C]-AP accumulation were 10 to 20-fold less potent than atropine (Table 2). These results indicate that the antacid action of *Baccharis trimera* active constituents is mediated mainly by inhibition of the cholinergic regulatory pathway of acid secretion.
secretion, a finding consistent with the reported inhibition of acid secretion in vivo stimulated by bethanechol or pentagastrin, but not by histamine in rats pretreated with the plant AE (Lapa et al., 1992).

Both the His- and CCh-induced $[^{14}C]$-AP uptake were significantly decreased by the flavonoids fraction (F9, 10–300 μg/ml) (Figs. 2 and 3) and diterpene 2 (F8, 10–100 μM) (Fig. 4). Diterpene 2 was the most active of all isolated compounds being 7-fold less potent than ranitidine and equipotent to atropine in reducing the $[^{14}C]$-AP uptake induced by the secretagogues (Table 2). In contrast, diterpene 1 (F10, 10–100 μM) was ineffective against the $[^{14}C]$-AP uptake stimulated by the secretagogues. Inhibition of the $[^{14}C]$-AP uptake induced by cholinergic and histaminergic stimulation in the presence of the flavonoids fraction and diterpene 2 indicate a possible inhibition of the H+, K+-ATPase, the final common step of acid secretion. Flavonoids (Murakami et al., 1999) and some diterpenoids identified in plants endowed with antacid and antiulcer activities such as scopadulciol and scopadulcic B isolated from Scoparia dulcis (Asano et al., 1990; Hayashi et al., 1991), and plectrimone A isolated from Plectranthus barbatus (Schultz et al., 2007) were shown to inhibit the gastric H+, K+-ATPase in vitro. The gastric proton pump, H+, K+-ATPase is a membrane-bound enzyme that upon stimulation translocates to the apical cell membrane and exchanges H+ for K+, at the expense of ATP hydrolysis (Sachs et al., 1976).

In control preparations of rabbit gastric membrane vesicles, the H+, K+-ATPase activity determined in the presence of 1 mM ATP was 29.0 ± 1.0 μmol Pi/mg protein/h (n = 8). Incubation of diterpene 2 (1 μM–1 mM) decreased the enzyme activity by 20% of control at the highest concentration, whereas the flavonoids fraction (10–1000 μg/ml) was inactive. Thus, inhibition of the gastric proton pump appears unlikely to account alone for the antacid activity of diterpene 2 and the flavonoids fraction, suggesting that these constituents exert their actions at intracellular sites upstream of the proton pump. The target sites of these actions and the mechanisms involved remain to be determined.

When tested at the same concentrations, none of the remaining fractions affected significantly the H+, K+-ATPase activity in vitro, except F7 which decreased the enzyme activity by 20–30% at all concentrations, indicating a non-specific action. In comparison, the proton pump inhibitor omeprazol (10 nM–1 mM) inhibited the H+, K+-ATPase activity with an IC50 = 10 μM.

Gastric ulcers result from a predominance of aggressive factors (acid secretion, Helicobacter pylori, stress, use of nonsteroidal antiinflammatory agents and ethanol) over protective mechanisms (mucus–bicarbonate, prostaglandin release, mucosal microcirculation) (Ham and Kaunitz, 2007). Gastric mucosal lesions induced by ethanol, stress or ischemia/reperfusion are accompanied by the decrease of gastric blood flow, increase of proinflammatory cytokine mediators, generation of reactive oxygen species, and the impairment of superoxide dismutase and protective antioxidative mechanisms (Kwiecien et al., 2003). Phenolic compounds such as plant-derived flavonoids and chlorogenic acid isomers have been reported to present gastroprotective effects unrelated to inhibition of acid secretion and exhibit healing of gastric ulcers by increasing the gastric mucosal blood flow through a local release of nitric oxide (NO), and by the release of calcitonin gene related peptide (CGRP) from sensory afferent nerves (Zayachkivska et al., 2005). Phenolic compounds also present free radical scavenging effects in vitro and are believed to exert antioxidant actions in vivo protecting against lipid peroxidation (Hamazu et al., 2006; Izuta et al., 2009). However, the latter possibility has been questioned because phenolics are hardly absorbed in the gut and are unlikely to reach the necessary concentrations in target tissues to influence oxidative stress (Crozier et al., 2009). Recent evidence indicates that metabolites of phenolics may affect different intracellular signaling mechanisms that are crucial for cellular functions which may be related to some of the reputed beneficial effects of natural products (Crozier et al., 2009). Whether this also applies to the active compounds of Baccharis trimera remains to be examined.

### 4. Conclusions

The data reported here indicate that the AE of Baccharis trimera presents active constituents that inhibit acid secretion by acting predominantly on the cholinergic regulatory pathway. The plant extract also contains constituents that exert moderate inhibition of the histaminergic regulatory pathway of acid secretion, as well as of the gastric H+, K+-ATPase. Altogether these active compounds appear to provide effective inhibition of acid secretion in vivo, which may explain the reputed antulcer activity of the plant extract. Nonetheless, the presented data do not exclude possible additional gastroprotective effects related to a local increase of gastric microcirculation induced by the plant phenolic constituents, and/or actions of phenolic metabolites on intracellular signaling pathways.
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References


