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Essential oils from *Baccharis* species (Asteraceae) have anti-inflammatory effects for human cells

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Constituents of the essential oils from the aerial parts of *Baccharis articulata* (Ba), *Baccharis genistelloides* subsp. *crispa* (Bc), *Baccharis dracunculifolia* (Bd) and *Baccharis gaudichaudiana* (Bg), Asteraceae, obtained by hydrodistillation and analyzed by gas chromatography (GC)/mass spectrometry (MS) and GC/flame ionization detector (FID) were identified. Also, their anti-inflammatory potential, focusing their immunomodulatory activity using flow cytometric analyses and AgNOR profiles, and anti-chemotactic properties, conducted using a Boyden's chamber system, were investigated. Fifty-eight compounds were characterized, representing 66.7% of the total components detected. The major constituents identified were spathulenol (in Ba, Bd and Bg), τ-gurjunene (in Bg) and palustrol (in Ba). The data presented herein showed that the *Baccharis* essential oils included in this study were inert for human resting lymphocytes, while all but Ba's inhibited significantly the proliferation of their phytohemagglutinin-stimulated counterparts. In addition, only the essential oil of Bd inhibited significantly the casein-induced human granulocyte chemotaxis. This is the first report concerning the potential diversity of anti-inflammatory activities of the essential oils of *Baccharis* plants on human cells responsible for the host defense mechanisms that may be of benefit in intervening with immune disorders associated or not with inflammatory conditions.

Keywords: *Baccharis*; Asteraceae; essential oil composition; spathulenol; immunomodulation; chemotaxis

Introduction

Baccharis L. is a large, strictly American genus of the Asteraceae family with 350 or more known species, some of which are commonly named in Brazil as 'carquejas'. Their aerial stems have been used by indigenous populations throughout Americas and in folk medicine, both internally and externally, from losing weight purposes (1) or diabetes (2) to alleviate or even cure a rather diverse list of disturbances, including diarrhea and dysentery (3), colds, sore muscle and muscle cramps, catarrhs, fever, edema, sores, dizziness, anemia, gastrointestinal disorders including stomach aches and ulcers (4), rheumatism, liver diseases, or wounds (2).

A peculiar characteristic of the *Baccharis* genus is the relative abundance of essential oil (EO) that can be obtained from its aerial parts. More than 3000 EOs are known, and at least 10% of them have commercial importance in the cosmetic, food and pharmaceutical industries as medicines or their coadjutants to promote medicine absorption, increasing penetration in the epidermis due to its lipophylic characteristics (5).

As part of a study of native 'carquejas' that grow in South Brazil, the main compounds present in the volatile oils of *Baccharis articulata* (Lam.) Pers., *Baccharis genistelloides* subsp. *crispa* (Spreng.) Joch. Müll, *Baccharis dracunculifolia* D.C. and *Baccharis gaudichaudiana* D.C. were identified. Furthermore, subsets of human peripheral blood leukocytes have been used to investigate whether these complex mixtures have the potential to be used as supplements or to enrich pharmaceutical preparations as an approach for combating inflammatory conditions. The selection of these four species was based on their medicinal uses not only in Brazil but in South America (6, 7) for the treatment or relief of symptoms related to inflammation.

Experimental

Chemicals

Unless otherwise specified, all chemicals used in this work were purchased from Sigma or Merck (São Paulo, SP, Brazil). Dexamethasone disodium phosphate

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(DEXA) was purchased from Aché Pharmaceutical Laboratories, Campinas, Brazil.

Plant material

All plants were collected in their native areas of Paraná State, Brazil, and identified by I.J.M. Takeda and N.I. Matzenbacker. *Baccharis articulata* was from Campo Magro (25°22'S and 49°27'W) and harvested in September 2001; *B. dracunculifolia* from Vila Velha (25°05'S and 50°09'W), February 2002; *B. genistelloides* subsp. *crispa* from Campo Largo (25°27'S and 49°31'W), February 2002; and *B. gaudichaudiana* from Inácio Martins (25°34'S and 51°04'W), October 2001. Vouchers of each species have been deposited in the herbarium of the Biosciences Institute, Federal University of Rio Grande do Sul, under the following series: *B. gaudichaudiana* – ICN 122943, *B. genistelloides* subsp. crispa – ICN 122944, *B. articulata* – ICN 122945 and *B. dracunculifolia* – ICN 122946.

Essential oil extraction

The samples (250 g) were subjected to 4 hours hydrodistillation using a Clevenger-type apparatus. The recovered oils were transferred to Eppendorf tubes, dried over anhydrous sodium sulfate powder, which was removed with the sediment after spinning at 400*g*, 4°C for 5 minutes. The oils were kept at −25°C in sealed brown vials until use.

GC/MS essential oil analyses and compounds' identification using apolar (HP-5 MS) and polar (ZB-Wax) column

Gas chromatography (GC) analyses were carried out in an Agilent 6890N gas chromatograph interfaced to a 5975 selective mass spectroscopy (MS) detector and 7683B automatic injector. The chromatographic separation was performed using J&W Scientific HP-5 MS, 5%-(phenyl)-methylpolysiloxane capillary column, measuring 30 m in length, with a 0.25-mm internal diameter and 0.25-µm film thickness and Phenomenex ZB-Wax, polyethylene glycol capillary column, measuring 60 m in length, with a 0.25-mm internal diameter and 0.25-µm film thickness. The temperature programming condition for HP-5 MS column was 60–240°C at 3°C/minute. Helium at 1.0 mL/minute was used as a carrier gas; 1.0 µL of oil injection; 220°C injector temperature; 250°C detector temperature; 1:50 splitting ratio; 70 eV ionization energy; 50–600 amu scan range. The temperature programming condition for ZB-Wax column was 45°C (6 minutes), 3°C/minute to 90°C, 5°C/minute to 180°C (16 minutes). Helium at 1.0 mL/minute was used as a carrier gas; 1.0 µL of oil injection; 220°C injector temperature; 240°C detector temperature; 1:50 splitting ratio. The constituents of the oils were identified by comparison of their MS data with corresponding data obtained from the NIST 2007 computerized MS-data bank and literature (8–11). A standard solution of *n*-alkanes (C8–C18 + C20) was injected in the same conditions of the samples in order to calculate the retention index (RI) and provide additional identification criteria.

GC/FID relative abundance determination

GC analyses were carried out in an Agilent 5890 gas chromatograph interfaced to a flame ionization detector (FID). The chromatographic separation was performed using J. &W. Scientific HP-5 MS 5%-(phenyl)-methylpolysiloxane capillary columns measuring 30 m in length, with a 0.25-mm internal diameter and 0.25-µm film thickness. The temperature programming condition was 60–240°C at 3°C/minute initial temperature. Helium at 1.0 mL/minute was used as a carrier gas; 1.0 µL of oil injection; 220°C injector temperature; 250°C detector temperature; 1:50 splitting ratio. The constituents of the oils were identified by comparison of their RI obtained in GC/MS analysis and literature data (8–11).

Human leukocyte isolation and fractionation

The protocol used for human peripheral blood collection has been approved by the Health Sciences Ethical Committee of the Federal University of Paraná, CEP/SD, No. 039.SI003/04-01, which is in accordance with the Declaration of Helsinki. Isolation of human leukocytes and their mononuclear (MNC) and granulocyte (GNC) fractions were obtained essentially as described elsewhere (12). After fractionation, the leukocyte subsets were kept in RPMI 1640 medium (Himedia Laboratories; Mumbai, India) supplemented with 10% fetal calf serum (FCS; Cultilab, Campinas, Brazil), 2 mM L-glutamine, 100 IU/mL penicillin and 50 µg/mL streptomycin sulfate (full medium).

Morphological studies

Where morphology was accessed, 8×10^4 cells cytocentrifuged using a Cytopro^{TM} instrument (Wescor, Utah, USA) were stained with May–Grunwald–Giemsa. At least 200 cells per assay were observed and differentiated for each experiment under immersion oil light microscopy. The MNC fraction contained lymphocytes (>90%) and monocytes, while >95% of the GNC fraction were neutrophils.

Cell viability and toxicity

The EOs (10^{-5}–100 µL/mL) were diluted in ethanol or dimethyl sulfoxide (DMSO), and their toxic effects on leukocyte subpopulations evaluated after exposure for 2 and 5 hours for GNC, and four and five days for MNC, respectively, at 37°C using the Trypan Blue exclusion test. Viability was taken as the percentage of the cells not incorporating the dye, counted in a

hemocytometer after directly diluting the cell suspension with 0.4% Trypan Blue solution.

Immunomodulation assays

The methodology used to investigate the effects of the EOs of *Baccharis* species on the proliferation and blastic transformation of peripheral human lymphocytes was essentially based on flow cytometric analyses fully described elsewhere (12, 13) with some modifications. Briefly, increasing doses (10^{-4}–10^{-2} μL/mL) of the EOs in ethanol were added to MNC (10^6) before or after culture as follows. Before stimulation: MNC were incubated with EOs for 24 hours at 37°C. Afterwards, the cells were washed with phosphate-buffered saline (PBS) and further incubated either in RPMI 1640 full medium alone or in medium enriched with 10% phytohemagglutinin-conditioned medium (PHA) for the next four days. After stimulation: MNC were first incubated in RPMI 1640 full medium enriched with 10% PHA for three days at 37°C to induce lymphocyte clonal expansion; then, increasing doses of the EOs (10^{-4}–10^{-2} μL/mL) were added and the cultures re-incubated for further two days at 37°C. The results are expressed as a proliferation index (IP) or as a percentage of blastic transformation ± standard error of mean (SEM), both resultant from the flow cytometric studies using the gating strategy already described (12,13).

Silver staining

The argyrophilic proteins of the nucleolar organizer regions (AgNOR) staining was carried out under immersion oil on cytospun preparations and was assessed numerically and morphologically according to the procedures first described by Shome and Khurana (14) and slightly modified by Florão and colleagues (13). The results are presented as the mean number of AgNOR per cell ± SD.

Migration assay

Granulocyte chemotaxis was measured using the Boyden's chamber method essentially as described elsewhere (15). Briefly, GNC (2×10^5) were exposed to the EOs (10^{-4}–10^{-2} μL/mL) or dexamethasone (DEXA; 10^{-3} M) for 30 minutes at 37°C, washed with PBS, and then stimulated to migrate towards 0.5% casein solution for 90 minutes at 37°C. In some experiments, cells were stimulated to migrate towards EOs directly placed into the lower chamber compartment. The results are expressed as the mean% ± SEM of migrated cells in relation to untreated cells, normalized at 100%.

Statistical analyses

Group data were analyzed by the Student's *t*-test or analyses of variance (ANOVA) followed by Turkey HSD-α=0.05 whereas necessary using the InStatSoft software. Differences were considered significant at $p \leq 0.05$.

Results

Chemical composition of the essential oils

In this work, the twelve major constituents, representing from 33.0% to 75.9% of the total EO components, obtained from the aerial parts of four species of the *Baccharis* genus that grow in South Brazil were characterized (Table 1). Yielding 0.1%, 0.5%, 0.6% and 0.8% (w/w), respectively, for *B. gaudichaudiana*, *B. articulata*, *B. genistelloides* subsp. *crispa* and *B. dracunculifolia*, their composition, apart from *B. gaudichaudiana*, was similar to other plants of the genus (7–11, 16) as expected, with the predominance of oxygenated sesquiterpene derivatives. Sesquiterpene hydrocarbons were represented mainly by τ-gurjunene followed by 2-*epi*-β-funebrene. GC/FID and GC/MS analyses showed that while spathulenol predominated in all species, palustrol was the major constituent of *B. genistelloides* subsp. *crispa*.

Cytotoxicity

Using the Trypan Blue exclusion assay, the tests performed with increasing doses of the EOs of *B. dracunculifolia* (90.0±4.2%), *B. genistelloides* subsp. *crispa* (92.7±1.8%) and *B. gaudichaudiana* (97.3±0.9%) showed no toxicity to human GNC up to 10^{-2} μL/mL independent of the incubation period (Figure 1, A1 and A2) when compared with untreated populations (controls). After 5 hours incubation at that dose, however, a significant drop in GNC viability was observed for the cells exposed to *B. articulata* EO (Figure 1, A1), with only 77.6±3.4% of the cells recovered. No toxicity was also observed for MNC up to the dose of 10^{-2} μL/mL after three or five days exposure, with 79.0±5.1%, 83.5 ±5.8%, 90.0±2.3% and 88.3±3.2% of viable cells, respectively for *B. articulata, B. dracunculifolia, B. genistelloides* subsp. *crispa* and *B. gaudichaudiana* (Figure 1, B1 and B2), when compared with the control's viability (86.0±4.2%).

In addition, May–Grünwald–Giemsa stained slides of these populations showed no cell morphology alterations. At higher doses, however, a significant decay in the viability of both MNC and GNC populations was observed, with less than 75% of the cells remaining alive. It is worth noting that simultaneous addition of PHA to the medium containing MNC improved cell survival even at 10^{-2} μL/mL, with >90% of the cells not incorporating the Trypan Blue dye after five days (data not shown).

Table 1. Major components of the essential oils of *Baccharis articulata* (Lam) Pers. (Ba), *Baccharis dracunculifolia* D.C. (Bd), *Baccharis gaudichaudiana* D.C. (Bg) and *Baccharis genistelloides* subsp. *crispa* (Spreng.) Joch. Müll (Bc), Asteraceae.

Compound	RI_{Calc} ZB-WAX	RI_{Calc} HP5	RI_{Lit}	Relative abundance (%) Ba	Bd	Bg	Bc	Ref.
2-*epi*-β-Funebrene	1603	1420	1417	5.5	1.2	ND	ND	8, 11
α-Amorphene	–	1486	1489	ND	1.6	ND	1.3	8
10,11-Epoxy-calamenene	1487	1500	1500	1.8	1.6	ND	ND	8
β-Bisabolene	–	1514	1513	ND	1.4	ND	0.4	8, 10
trans-Calamenene	1767	1525	1522	2.3	ND	ND	ND	8, 9
Palustrol	2044	1572	1563	ND	0.4	ND	29.5	8, 11
Spathulenol	2048	1591	1575	30.6	47.9	41.0	1.8	8, 9, 11
τ-Gurjunene	2107	1601	–	4.9	ND	34.9	ND	
Humulene epoxide II	–	1613	1607	0.3	1.2	ND	ND	8
Caryophylla-4(12).8(13)-dien-5-ol	2074	1643	1644	1.6	ND	ND	ND	8, 11
α-Muurolol	–	1644	1645	ND	1.5	ND	ND	8
epi-α-Bisabolol	–	1688	–	1.0	0.6	ND	ND	

Notes: RI_{Lit}, literature retention index; RI_{Calc}, calculated retention index; ND, not detected.

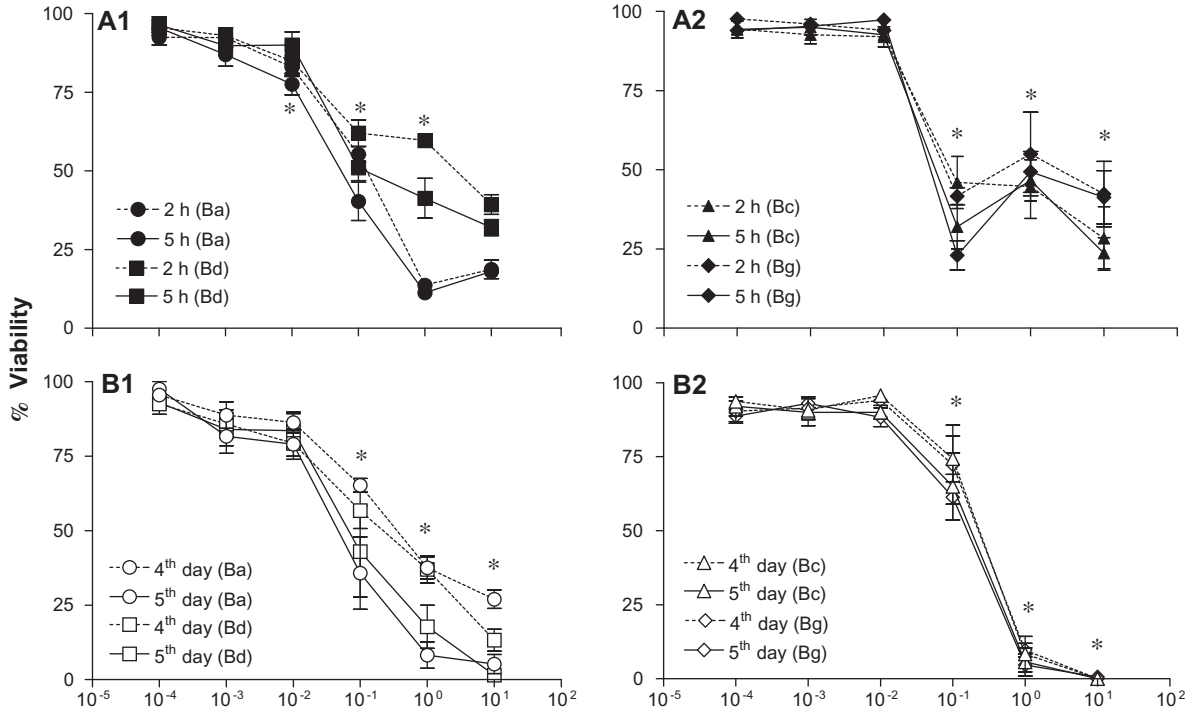

Figure 1. Effects of the essential oils extracted from the aerial parts of *Baccharis articulata* (Ba), *Baccharis dracunculifolia* (Bd), *Baccharis genistelloides* subsp. *crispa* (Bc) and *Baccharis gaudichaudiana* (Bg) on peripheral leukocytes survival. Granulocytes (A) and mononuclear cells (B) were exposed at 37°C to the indicated doses of the essential oils (μL/mL) for 2 and 5 hours, or four and five days, respectively, and their viability evaluated using the trypan blue exclusion test. Each point represents the mean percentage ± SEM of viable cells. $^{*}p<0.001$; n=3–6.

Immunomodulatory activity

To investigate the effects of the EOs on resting (Figure 2A) and proliferating lymphocytes (Figure 2B), MNC were exposed to doses ranging from 10^{-4} to 10^{-2} μL/mL in different conditions and analyzed by flow cytometry.

A dose-related drop in the cell proliferation index not related to toxicity (Figure 2C) was observed, but only for the PHA-stimulated populations when compared with PHA-control values (3.5±0.4; n=4), which was significant for the EOs of *B. genistelloides* subsp. *crispa* (2.2±0.2; n=3), *B. gaudichaudiana* (2.1±0.1; n=4) and *B. dracunculifolia* at 10^{-2} μL/mL (2.1±0.4;

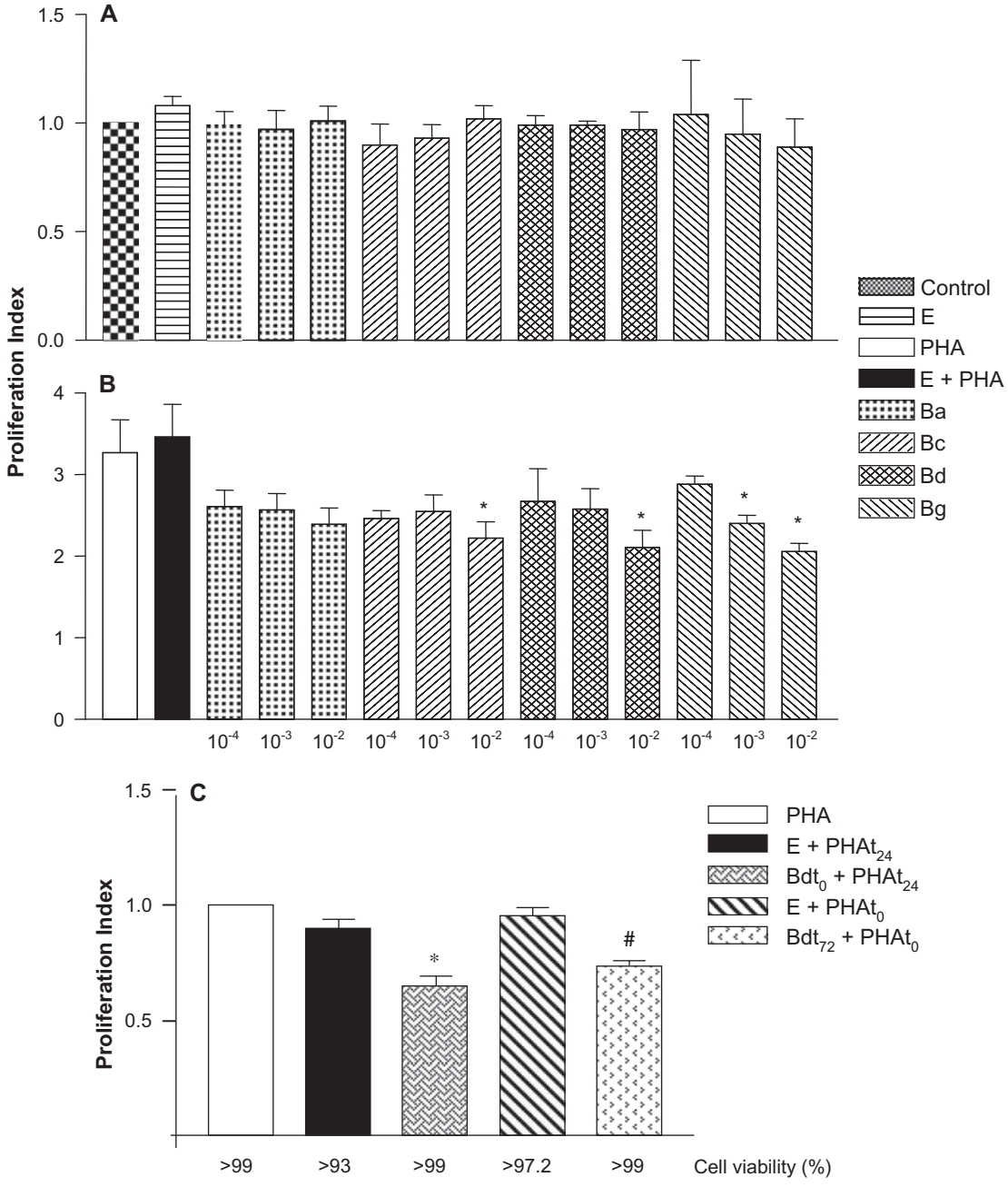

Figure 2. Effects of the essential oils from *Baccharis articulata* (Ba), *Baccharis genistelloides* subsp. *crispa* (Bc), *Baccharis dracunculifolia* (Bd) and *Baccharis gaudichaudiana* (Bg) on the proliferation of human lymphocytes. Human mononuclear cells were exposed to the indicated doses of the essential oils diluted in ethanol (E) for five days, at 37°C, and 5% CO_2 (A). In some experiments, phytohemagglutinin conditioned medium (PHA) was added (B). In other series, the essential oil of *B. dracunculifolia* at 10^{-2} µL/mL was added at the setting up period (t_0) or three days (t_{72}) after PHA stimulation (C). Trypan blue exclusion test was used to verify cell viability after five days incubation. Each bar represents the proliferation index ± SEM resultant from the flow cytometric studies. $^{*}p \leq 0.05$, $^{\#}p < 0.01$, both in relation to (E+PHA)-treated control.

n=4). For *B. gaudichaudiana*, the inhibitory effect was far more effective, and was significant even at 10^{-3} µL/mL (2.4±0.1; *n*=4). In addition, a significant lower dose-related number of blasts was detected in the lymphoblast region of the flow cytometer for the same EO-exposed PHA-treated cells (Table 2).

Cytospun preparations confirmed the differences in the percentage of lymphocytes and lymphoblasts present in each population (Table 3). Moreover, the block in cell proliferation for the PHA-stimulated treated populations was also evident by the lower numbers of silver nucleolar organizer regions staining regions

Table 2. *In vitro* effects of the essential oils from different species of the genus *Baccharis* on blastic transformation of human lymphocytes.

	% of Blasts (R2)		
Control	17.8±2.8		
+ PHA	80.2±2.8*		
Ethanol (20 μL/mL)	16.2±3.4		
+ PHA	83.5±4.2*		
Essential oil (μL/mL)	10^{-4}	10^{-3}	10^{-2}
Baccharis articulata	19.7±4.6	17.8±4.0	18.4±3.8
+ PHA	80.6±2.4*	81.7±1.8*	81.7±1.8*
Baccharis genistelloides subsp. *crispa*	24.2±4.2	22.0±3.4	25.8±7.3
+ PHA	67.7±4.5#	67.3±5.1#	67.0±5.3#
Baccharis dracunculifolia	15.1±4.9	17.5±3.7	19.8±3.6
+ PHA	80.9±2.9*	80.4±3.3*	81.5±2.5*
Baccharis gaudichaudiana	19.8±2.0	19.1±7.8	19.5±6.8
+ PHA	72.2±1.4#	69.3±4.0#	64.4±9.1#

Notes: Human mononuclear cells were exposed to the indicated doses of the essential oils for five days, at 37°C and 5% CO_2. In some experiments, phytohemagglutinin-conditioned medium (PHA) was added. Each value represents the percentage ± SEM of cells detected in the flow cytometry region R2 in relation to the control population (R1) from at least three independent experiments, each one run in triplicates. *$p<0.05$; #$p<0.01$ in relation to respective controls.

Table 3. Effects of the essential oils from different species of *Baccharis* on human lymphocyte morphology.

	Lymphocytes (%)		Blasts (%)			
Control	85		15			
+ PHA	30		70			
Ethanol (20 μL/mL)	89		11			
+ PHA	15		85			
Essential oil (μL/mL)	10^{-4}		10^{-3}		10^{-2}	
	Lym (%)	Blasts (%)	Lym (%)	Blasts (%)	Lym (%)	Blasts (%)
Baccharis articulata	99	01	97	03	98	02
+ PHA	06	94	09	91	09	91
Baccharis genistelloides subsp. *Crispa*	–	–	–	–	–	–
+ PHA	43	57	44	56	45	55
Baccharis dracunculifolia	94	06	96	04	95	05
+ PHA	22	78	18	82	19	81
Baccharis gaudichaudiana	–	–	–	–	–	–
+ PHA	34	66	42	58	46	54

Notes: Human peripheral mononuclear cells were treated with ethanol or the indicated doses of different *Baccharis* spp. Essential oils for five days, at 37°C and 10% CO_2. In some experiments, 1% phytohemagglutinin conditioned medium (PHA) was added 24 hours before the essential oils. Each value represents the mean percentage ± SD of the different cell types observed by immersion oil microscopy in cytopreparations after May–Grunwald–Giemsa staining.

(AgNOR) found in cytospun preparations (Figure 3), ranging from 2.8±0.2 AgNOR/cell (PHA+E control population) to 1.71±0.28 for *B. articulata* at 10^{-2} μL/mL; 1.80±0.21 and 1.76±0.05, for *B. genistelloides* subsp. *crispa*, respectively, at 10^{-3} and 10^{-2} μL/mL as it was 1.78±0.34 and 1.80±0.01 for *B. dracunculifolia*. Finally, for *B. gaudichaudiana*, the values were 1.81 ±0.08 and 1.74±0.13 AgNOR/cell, respectively, to the doses of 10^{-3} and 10^{-2} μL/mL.

In order to clarify whether the EOs were interfering with cell activation or with the proliferation that occurs after activation, MNC cells were, first, treated with 10^{-2} μL/mL of the EO of *B. dracunculifolia* for 24 hours, followed by addition of PHA-conditioned medium and re-incubated for four days. In another series of experiments, MNC were exposed to PHA-conditioned medium for three days; then, 10^{-2} L/mL of the EO of *B. dracunculifolia* were added followed by two more days of incubation. As already showed in Figure 2A, significant inhibition of cell proliferation was observed in both conditions.

Anti-chemotactic activity

To explore whether the EOs of the four species would interfere with the inflammation process, two series of experiments were performed. First, human GNC were induced to migrate towards doses from 10^{-4} to 10^{-2} µL/mL of EOs placed into the lower chamber compartment (Figure 4A).

In a second approach, GNC were first exposed to the same doses of the EOs and then induced to migrate towards a casein gradient, a potent chemoattractant for human neutrophils (Figure 4B), the main component of this fraction as stated before. Only under this last circumstance, inhibition of GNC migration was observed for the EOs of *B. articulata* and *B. dracunculifolia*, reaching maximum significance at 10^{-2} µL/mL, with 53.9±12.5 and 41.7±10.2, respectively, of the input cells recovered. However, it is important to note that the EO of *B. genistelloides* subsp. *crispa* at 10^{-2} µL/mL was toxic for GNC as presented earlier (Figure 1A). Therefore, only the EO of *B. dracunculifolia* exerted a significant anti-chemotactic effect upon human GNC, which was better than that showed by DEXA, for which 59.0±7.2% of the cells was retained in the upper chamber compartment.

Discussion

In the last few years, many articles became available concerning the pharmacological properties of the EOs of the *Baccharis* genus, particularly showing a broad spectrum of antimicrobial activities against several infective strains of bacteria, fungi, virus and protozoan parasites (17–21), along with toxicity (2, 20) and insect repellence (22). However, their anti-inflammatory

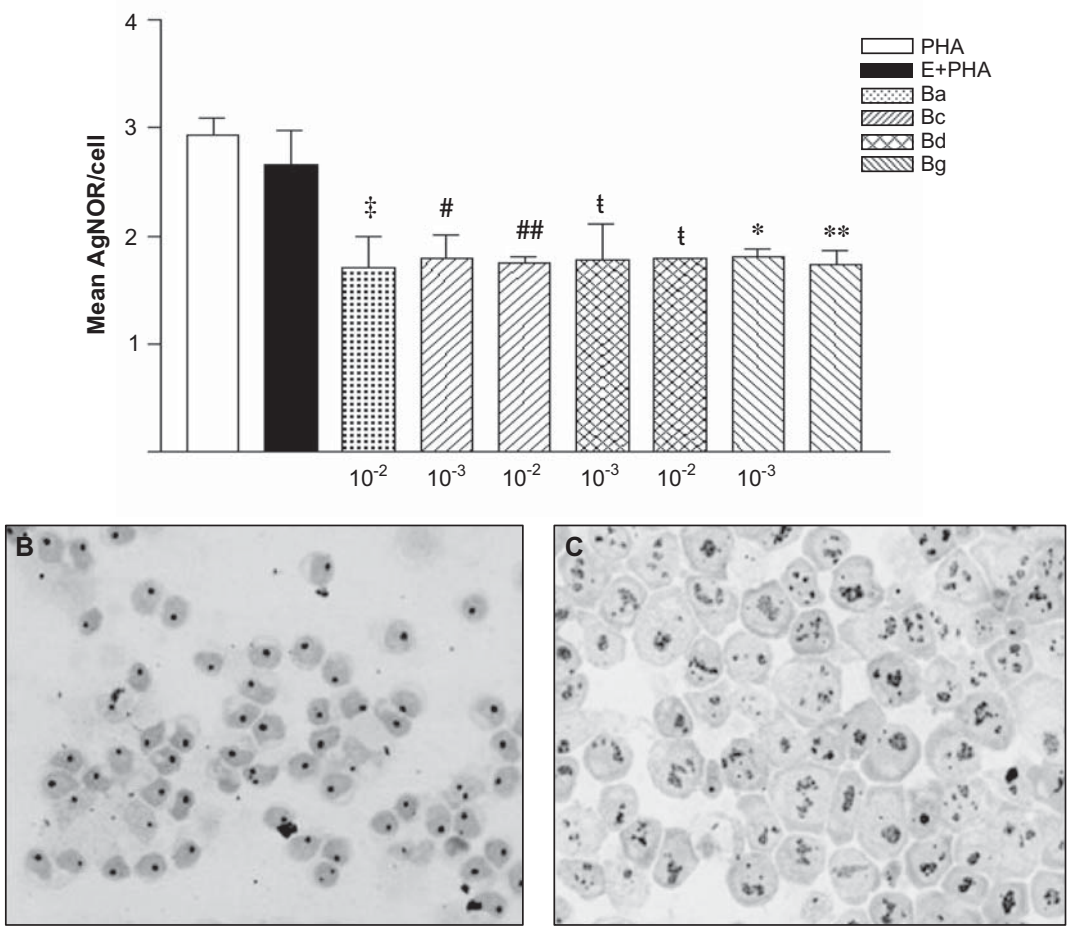

Figure 3. Effects of the essential oils of *Baccharis articulata* (Ba), *Baccharis genistelloides* subsp. *crispa* (Bc), *Baccharis dracunculifolia* (Bd) and *Baccharis gaudichaudiana* (Bg) on the number of AgNOR of lymphocytes. Human mononuclear cells were exposed to the indicated doses of the essential oils diluted in ethanol (E) for five days, at 37°C, 5% CO_2 in presence of phytohemagglutinin-conditioned medium (PHA). The number of AgNOR in each cell (A) was carried out under immersion oil on cytospun preparations and was assessed numerically and morphologically for resting (B) and PHA-stimulated (C) populations. Each bar represents the mean number of AgNOR/cell ± SD. $^{‡}p$=0.043, $^{\#}p$=0.018; $^{\#\#}p$=0.0085; ^{ŧ}p=0.036; $^{*}p$=0.011; $^{**}p$=0.0099, all in relation to (E+PHA)-treated control.

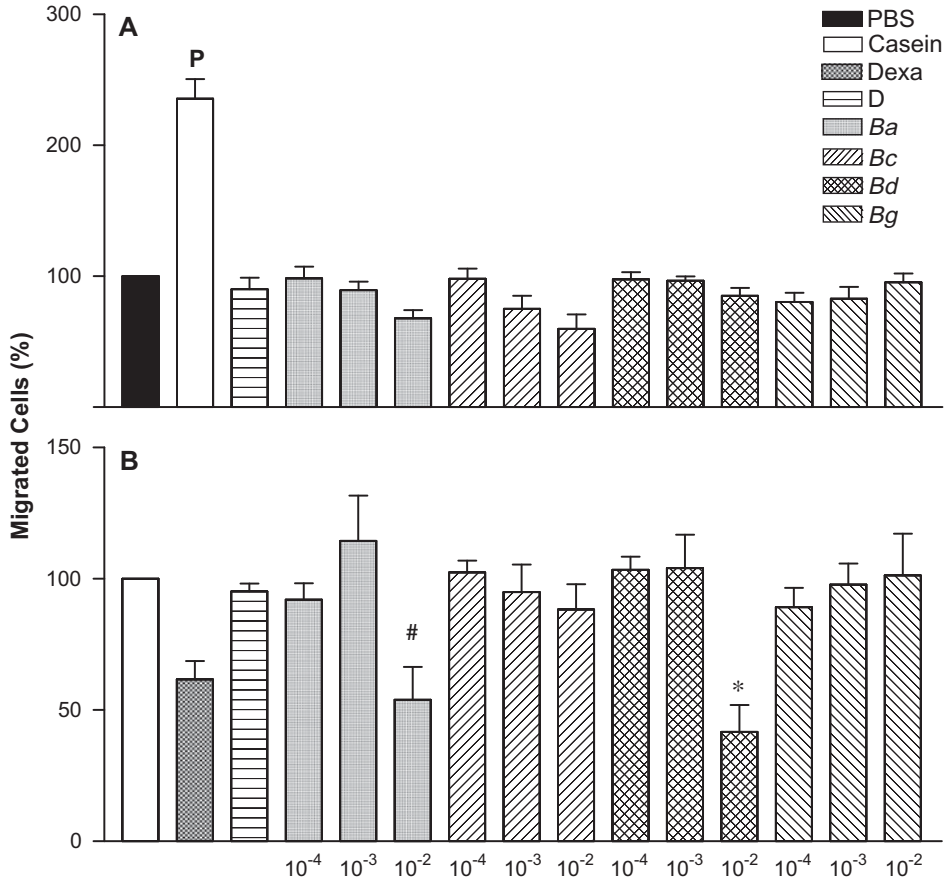

Figure 4. Effects of the essential oils from *Baccharis articulata* (Ba), *Baccharis genistelloides* subsp. *crispa* (Bc), *Baccharis dracunculifolia* (Bd) and *Baccharis gaudichaudiana* (Bg) on the migration of human granulocytes. Peripheral blood granulocytes were exposed to the indicated doses (μL/mL) of the essential oils (A) placed in the lower compartment of a Boyden chamber for 90 minutes at 37°C, in which the compartments were separated by a 5-mm pore size polycarbonate filters. (B) In some experiments, the cells were first pretreated with the indicated doses of the essential oils, dexamethasone (DEXA; 10^{-3} M) or dimethyl sulfoxide (DMSO) (D; 20 μL/mL) for 30 minutes at 37°C, before induced to migrate toward a 0.5% casein gradient. Each column represents the mean percentage ± SEM of cells recovered from the lower compartment in relation to untreated cells, normalized at 100%. $^{*}p<0.01$; $^{**}p<0.05$, all in relation to respective untreated populations; n=3–8.

properties have been poorly explored. Within this context, three articles referring to the potential of these EOs in interfering with the inflammatory response could be found in the literature by the time this manuscript was prepared (4, 6, 23), and two of them were related to the beneficial antiulcer effects of the EO of *B. dracunculifolia* in animal models (4, 23), corroborating with this activity described earlier for its crude extract.

As the use of 'carquejas' in folk medicine is generally related to conditions involving the inflammatory response, we have explored the possibility that these rich mixtures would present anti-inflammatory potential. To achieve this, we have chosen *in vitro* assays involving, on the one hand, peripheral human mononuclear cells, composed mainly by lymphocytes, the major protagonists of the immunomodulation processes, and on the other hand, human granulocytes, particularly neutrophils, the key components of the inflammation reactions.

First, we examined the ability of these oils in interfering with the immune response. Human peripheral MNC, which are mainly composed by lymphocytes, were incubated with increasing doses of EOs in two distinct situations: before activation, in which the cells were exposed only to increasing doses of the EOs; and three days after PHA activation, in which the majority of the cells was already undergoing mitosis (lymphoblasts). In the results presented in Figure 2, it is clear that, apart from the EO of *B. articulata*, pre-incubation with the other EOs affected significantly only replicating cells, which were stimulated by PHA, a well known potent mitogen for T cells. As these inhibitory effects were not related to cytotoxicity, as recently described for an aqueous extract prepared from *B. trimera* (24), the results thus indicate that the EOs

were not able to activate human lymphocytes in the *in vitro* conditions of the assays. In contrast, they showed, at least for the EO of *B. dracunculifolia*, competency for inhibiting PHA activation or, alternatively, preventing the proliferation of PHA-activated lymphocytes. Within this context, an immunomodulatory action in mice has been reported for extracts prepared from leaves and roots of *B. dracunculifolia* (25). The inhibitory effects found for PHA-activated lymphocytes were also consistent with the lower number of blasts (Table 2) and AgNORs (Figure 3) observed for these populations. It is worth noting that AgNORs are loops of DNA transcribed into ribosomal RNA that undergo a rise in number and variations in size and shape in conditions that traditionally involve enhanced cell proliferation and rRNA transcription. As such, they have been used as markers of cell proliferation (26). Whatever it may be, our data showed a feature of these complex mixtures not reported before for human cells, in which they were competent to interfere with the proliferation of actively dividing lymphocytes.

As the immune system and the inflammatory response work cooperatively and synchronically to eliminate and keep the organism free of invaders, and migration towards injury sites is one the major steps taken by circulating GNC in an inflammatory process, we also investigated whether the EOs would interfere with the human leukocyte migration. The results indicate that the EOs of the *Baccharis* genus studied were not efficient by themselves for promoting any directional movement of human GNC. However, when casein was used as chemoattractant, many cells previously exposed to the EOs, which was significant for *B. dracunculifolia*, were retained in the upper chamber. It is of interest to note that casein is a taxin well known by its potent ability to attract human neutrophils, and that pre-treatment with DEXA can efficiently block this directional movement (27). Therefore, the ability of this particular EO in inhibiting the mobilization of human neutrophils was far more efficient than DEXA. These results are in agreement with a recent report that describes attenuation in the mobilization of neutrophils in an experimental inflammatory model of carrageenan-induced pleurisy in rats treated with *B*. *trimera* extract (24).

While the cellular and molecular mechanisms involved in this highly regulated cascade of events that plays a central role in host defense remain to be elucidated, the preliminary findings presented herein allow us to suggest that the plants of the *Baccharis* genus do have anti-inflammatory potency. Furthermore, among the species studied, *B. dracunculifolia* showed the best anti-inflammatory effects, which may be related to spathulenol, its most abundant constituent, which has recently shown an immunomodulatory effect by inhibiting the proliferation of human peripheral blood lymphocytes, inducing them into apoptosis (28).

No data concerning the anti-inflammatory potential of the EOs from the *Baccharis* species included in this study is available in the literature. Therefore, the overall results herein presented are relevant as, on the one hand, they have shown evidence that these complex mixtures contain a richness of principles that have crucial effects on cells that act in various steps of the inflammatory process. On the other hand, they must stimulate further investigations in order to establish the therapeutic usefulness of *Baccharis* plants. It will not be a surprise to find that these EOs may be a novel class of effective therapeutics with application in pharmaceutical preparations used for inflammatory conditions.

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