Analgesic, anti-inflammatory and antioxidant properties of *Buddleja globosa*, Buddlejaceae

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Abstract

Ethnopharmacological relevance: *Buddleja globosa*, known as "matico", is employed in Chile for wound healing.

Aim of the study: To validate the traditional use of the crude drug through *in vivo* and *in vitro* evaluation of the anti-inflammatory, analgesic and antioxidant properties of its extracts.

Materials and methods: Sequential hexane, dichloromethane, methanol and total methanol extracts were studied using bioguided fractionation. The following activities were investigated: analgesic (writhing test), oral and topic anti-inflammatory (paw- and ear-induced edema), free radical scavenging and antioxidant activities (1,1-diphenyl-2-picrylhydrazyl, DPPH, superoxide anion, lipid peroxidation and xanthine oxidase inhibition). Sodium naproxen, nimesulide, indomethacin were used as reference drugs for *in vivo*, quercetin and allopurinol for *in vitro* assays.

Results: A mixture of α- and β-amyrins was isolated from the hexane extract that showed 41.2% of analgesic effect at 600 mg/kg, inhibited by 47.7 and 79.0% the arachidonic acid (AA) and 12-deoxyphorbol-13-decanoate (TPA)-induced inflammation at 3 mg/20 μL/ear, respectively. A mixture of β-sitosterol, stigmasterol, stigmastenol, stigmastanol and campesterol was isolated from the fraction CD4-N and β-sitosterol-glycoside from the fraction CD5-N, reducing TPA-induced inflammation by 78.2 and 83.7% at 1 mg/20 μL/ear, respectively. The fraction CD4-N at 300 mg/kg also showed analgesic activity (38.7%). The methanol extract at 600 mg/kg *per os* showed anti-inflammatory effect (61.4%), topic anti-inflammatory (56.7% on TPA) and analgesic activity (38.5%). Verbascoside and luteolin-7-*O*-glucoside were the major components of the methanol extract; apigenin 7-*O*-glucoside was also detected. Inhibition of superoxide anion, lipoperoxidation, and DPPH bleaching effect was found in the methanol serial and global extracts.

Conclusions: The present report demonstrate the analgesic and anti-inflammatory properties of *Buddleja globosa* and validate its use in Chilean traditional medicine.

Keywords: *Buddleja globosa*; Buddlejaceae; Analgesic; Anti-inflammatory; Antioxidant; Flavonoids; Phenylethanoids; Sterols; Triterpenoids

1. Introduction

The leaves of *Buddleja globosa* Hope (Buddlejaceae), known as "matico" in Chile are widely used in folk medicine because of their reputed wound- and gastric ulcer healing effects (Muñoz et al., 1981). Previous studies on "matico" describe triterpenes, diterpenes, phenylethanoids and flavonoids as main chemical constituents. Wound healing and anti-inflammatory evaluations on the hydroalcoholic extract of the aerial parts and dichloromethane root extract *in vitro* have been reported (Liao et al., 1999; Mensah et al., 2000, 2001; Houghton et al., 2005). Domestication studies have been also carried out for its protection and sustainable production (Vogel et al., 2002).

The aim of this study is the validation of the medicinal use of *Buddleja globosa* leaves and the search for analgesic, anti-inflammatory and antioxidant activities in the crude drug. Pain and inflammation are strictly related to wound healing (Das and Maulik, 1994) and free radicals increase during all these pathological process, extending inflammation and influencing wound healing.

The present investigation was undertaken to establish the analgesic, anti-inflammatory and antioxidant effects of a Chilean sample of *Buddleja globosa* through bioguided *in vivo* evaluation of serial extracts, to identify its main chemical constituents and to scientifically support *Buddleja globosa* properties, validating its medicinal use. For *in vivo* assays, topic and oral administration were used as in folk medicine. Herein we report the antioxidant, analgesic and anti-inflammatory effects and the main components of each active extract that account for the demonstrated activities.

2. Materials and methods

2.1. General experimental procedures

^{1}H and ^{13}C NMR were recorded in DMSO and CD$_3$OD at 300 or 400 MHz for ^{1}H and 75 or 100 MHz for ^{13}C with TMS as internal standard. GC–MS analysis was made on Fissons MB 800 mass spectrometer (MS) coupled with Hewlett-Packard mod. 5890 series II gas chromatograph (GC) equipped with a 15 m × 0.32 mm i.d. HP U-2 column, with 0.25 μm thickness film and NIST database. The initial oven temperature was held at 40 °C for 6 min, it was then increased at 7 °C/min up to 200 °C, at 15 psi with helium as carrier gas. HPLC–DAD analysis. Retention time and UV spectra were recorded in a HPLC Waters 486 equipment with a coupled UV–vis diode array detector, using a RP 18 column (150 mm × 4 mm). The solvent system used was acetonitrile:aqueous 0.1% formic acid (25:75). Detection: 365 nm, flow rate: 1.5 mL/min. IR spectra were measured in a PerkinElmer 1310 equipment in KBr. Column chromatography (CC) was performed using silica gel 60G (Merck 7734), thin layer chromatography (TLC) on silica gel GF254 (Merck 5554). Spots were detected under UV light and spraying with different reagents as Liebermann–Burchard, anisaldehyde and NP/PEG reagent.

2.2. Plant material

The aerial parts of *Buddleja globosa* Hope (Buddlejaceae), were collected in Temuco, IX Region, Chile, in November 2000. A voucher specimen is kept at the Herbarium of the Escuela de Química y Farmacia (SQF 22219), Universidad de Chile.

2.3. Extraction and isolation

Air-dried and powdered leaves (4.8 kg) were sequentially extracted at room temperature with *n*-hexane, CH$_2$Cl$_2$ (DCM) and MeOH, yielding after removal of the solvents under reduced pressure 114.3 g of HE (2.4%), 77 g of DCME (1.6%) and 413 g of EMAT1 (8.6%) extracts, respectively. A new amount of plant material (227 g) was extracted with MeOH at room temperature to afford the global MeOH extract (GME, 21.8 g, 9.6%), used for preliminary pharmacological assays. After evaluation and according to results, the active extracts (HE, DCME and EMAT1) were submitted to bioguided fractionation. The HE (92 g) was chromatographed on silica gel (CC) eluting with mixtures of hexane–DCM, DCM and DCM–MeOH and MeOH. Fractions eluted with hexane (25 g) were pooled together and subjected to a second CC over silica gel using hexane–DCM (90:10 v/v and 80:20 v/v) as the mobile phase to afford a mixture (281 mg) of β- and α-amyrins (3:2). Identification was performed by direct chromatographic comparison (TLC) with authentic compounds and by GC–MS in comparison with bibliographic data (Karliner and Djerassi, 1966; Backhouse et al., 1997).

The DCME (70 g) was defatted by dissolving the extract in a minimal amount of dichloromethane and stirring with hot methanol. After standing at 6 °C for 24 h the precipitate was filtered-off, and the solution taken to dryness to yield the DDCME (57 g). A part of the bioactive DDCME (47 g) was subjected to repeated CC, yielding two active fractions CD4-N (9 g), eluted with hexane:DCM (70:30) to DCM:MeOH (95:5) and CD5-N (20.3 g), eluted with DCM:MeOH (90:10 to 50:50). Fraction CD4-N (9 g) was submitted to CC yielding 41 mg of a triterpene mixture, 175 mg of a steroid mixture, 92.7 mg of β-sitosterol glucoside, eluted with hexane:EtOAc (95:5, 80:20 and 40:60, respectively). CD5-N contained mainly β-sitosterol glucoside (362 mg). The constituents of the steroid mixture were identified by GC–MS, in comparison with retention time (*t'R*) and Nist library database of standard sterols, as β-sitosterol (22.31 min) and stigmasterol (20.70 min) (major constituents) together with campesterol (19.62 min), stigmastanol (22.57 min) and ergosterol (24.16 min). The β-sitosterol glycoside was identified by its spectral data and comparison with an authentic sample (Backhouse et al., 1997).

The EMAT1 (20 g) was subjected to CC on silica gel, eluting with solvent mixtures of increasing polarity. From fractions eluted with DCM:EtOAc (40:60), β-sitosterol glucoside (20 mg) was obtained. Fractions eluted with DCM:EtOAc (20:80) and EtOAc 100% yielded verbascoside (350 mg), luteolin 7-*O*-glucoside (220 mg) and apigenin 7-*O*-glucoside (5 mg). Identification of apigenin 7-*O*-glucoside was performed by TLC

comparison and by HPLC (retention time 12.32 min and UV spectra λ_{max} 266, 336 mm) with authentic compound. Identification of verbascoside and 7-O-luteolin glycoside was performed by extensive ^{1}H NMR and ^{13}C NMR measurements including HMQC and HMBC experiments. Results were compared with reported data (Li et al., 2005; Wu et al., 2004; Harborne and Mabry, 1982). Reference spectra are available on request from the correspondence author.

2.4. Animals

All animal experiments were performed according to the ethical guidelines suggested by the "International Norms for the Biomedical Investigation with Animals", elaborated by the Council of International Organizations (1990) and the bioethics norms of the Commission of the Chilean Public Health Institute and Faculty of Chemical and Pharmaceutical Sciences. Pirbright guinea pigs (220–300 g) of both sexes were used for *per os* anti-inflammatory study and CF-1 mice of either sex (20–25 g) were used to assess the analgesic and topic anti-inflammatory effects. Efforts were made to minimize animal suffering and to reduce the number used. Animals were housed in a climate- and light-controlled room with a 12-h light/dark cycle, fasted overnight before the day of the assays, with free access to water.

2.5. Analgesic activity

The analgesic activity was evaluated in groups of 8 mice and 2 controls by intraperitoneal injection of 0.5 mL of 0.6% acetic acid. The analgesic effects were calculated by comparing the number of abdominal writhes of the treated and the control group, which only received the vehicle. The number of abdominal writhes of each mouse was counted for 30 min, beginning 5 min after acetic acid administration. Sodium naproxen was used as reference drug, showing 70.0% of analgesic effect at 12.5 mg/kg.

Mean pain percentage was calculated as: $\%P = [C_{sample}/C_{control}] \times 100$; where C sample is the mean writhes reached in sample-treated animals and $C_{control}$ (41.6 ± 3.79) is the mean writhes reached in control animal which received only the vehicle. The analgesic effect (An) was calculated according to the following equation: $\%An = 100 - \%P$ (Delporte et al., 2005).

2.6. Anti-inflammatory activity per os

For each oral dose, the anti-inflammatory activity was evaluated in groups of 12 guinea pigs, 10 for the sample and 2 for control, using the λ-carrageenan-induced paw edema. Paw volume was measured with an Ugo Basile plethysmometer (model 7150) 3 h after injecting 0.1 mL of sterile saline λ-carrageenan (1%).

Anti-inflammatory activity (A) was evaluated as: $\%A = [(\%I_c - \%I_s)/\%I_c] \times 100$; where $\%I_c$ is the mean inflammatory response in the control group receiving only the vehicle $(34.0 \pm 2.3\%$ paw volume increase), and $\%I_s$ corresponds to the mean inflammation in the sample-treated animals, expressed as: $\%I = [(V_f - V_i)/V_i] \times 100$; where V_f and V_i are

final and initial paw volumes, respectively (Backhouse et al., 1997). Sodium naproxen was used as reference drug at 4 mg/kg with 54.6% of maximum anti-inflammatory (Backhouse et al., 2002).

In oral anti-inflammatory and analgesic assays, dry extracts (GME, HE, DDCME and EMAT1) were administered 1 h before λ-carrageenan (Sigma), or acetic acid by means of gavage, suspended in saline arabic gum. Doses used in each *per os* assay were selected according to previous work on the same biological activity (600 and 300 mg/kg for extracts and fractions, respectively).

2.7. Topic anti-inflammatory activity

For each dose of the sample under study, the anti-inflammatory activity was evaluated in groups of 8 mice and 2 controls. Mice were treated with the sample and after 5 min they received 2 mg arachidonic acid (AA) or 2.5 μg 12-deoxyphorbol-13-decanoate (TPA) dissolved in 20 μL acetone. Control subjects received only AA or TPA at the same concentration. Sample and the inflammation agent (AA or TPA) were applied to the right ear and only acetone to the left. Mice were sacrificed by cervical dislocation and a 6-mm diameter section of the right and left ears were cut and weighed (Lloret and Moreno, 1995). Dermal anti-inflammatory activity (TA) was evaluated according to the following equation: $\%TA = [(W_c - W_s)/W_c] \times 100$; were W_c and W_s are the difference mean values of the weights of the right and the left ear sections of the control and the treated animals, respectively.

Doses used were selected according to previous work on the same biological activity (3 and 1 μg/20 μL/ear for extracts and fractions, respectively). Nimesulide (AA-induced oedema) and indomethacin (TPA-induced oedema) were used as reference drugs at the dose of 1 mg/20 μL/ear and 0.5 mg/20 μL/ear, respectively with 48.8 and 92.9% of anti-inflammatory effect (Delporte et al., 2005).

2.8. Statistical analysis

For all pharmacological assays, the drug-induced changes were statistically estimated using the Wilcoxon test for independent data (Hollander and Wolfe, 1999). The effects were considered significant for $P \leq 0.05$. The SEM values were calculated for the mean $\%I_c$ and $\%I_s$ values. I_c and I_s are the mean inflammation reached in the control and in the sample-treated guinea pigs, respectively, for the mean writhes constriction and for the mean weight ears in treated and untreated animals in each assay.

2.9. Xanthine oxidase activity

Xanthine and xanthine oxidase (XO) from cow's milk were purchased from Sigma Co. and the standard inhibitor allopurinol was obtained from Laboratorios Saval, Chile. Samples were dissolved in bi-distilled water and DMSO, not exceeding 1%. The extracts were evaluated at 50 μg/mL. Inhibition of the xanthine oxidase (XO) activity was measured spectrophotometrically at

290 nm as reported by Delporte et al. (2005). The inhibition percentage of xanthine oxidase activity (%I) was calculated as: $\%I = (A - B) - (C - D)/(A - B) \times 100$ where A is the XO activity without testing extract (total uric acid); B, the blank of A without XO; C, the enzyme activity with testing extract (residual uric acid); and D, the blank of C without the enzyme. Under our working conditions, the IC_{50} of the standard inhibitor allopurinol was 0.035 μg/mL (0.267 μM). For XO activity, the extract-induced changes were statistically determined with the Wilcoxon test for independent data (Hollander and Wolfe, 1999). Effects were considered significant for $P \leq 0.05$.

2.10. Superoxide anion

The enzyme xanthine oxidase is able to generate O_2 in vivo by oxidation of reduced products from intracellular ATP metabolism. The superoxide generated in this reaction sequence reduce the nitro blue tetrazolium dye (NBT), leading to a chromophore with maxima at 560 nm.

Superoxide anion scavengers reduce the generation of the chromophore. The activity was measured spectrophotometrically as reported by Masaki et al. (1995). Extracts and products were evaluated at 50 μg/mL. Values are presented as mean ± standard deviation of three determinations.

2.11. Peroxidation in erythrocytes

Studies on erythrocyte lipid peroxidation were carried out as described by De Azevedo et al. (2000) with slight modifications. Human red blood cells obtained from healthy donors were washed three times in cold phosphate buffered saline (PBS) by centrifugation at a relative centrifugal force (RCF) of $1917 \times g$. After the last washing, cells were suspended in PBS and its density adjusted to 1 nM haemoglobin in each reaction tube. The final cell suspensions were incubated with different concentrations of the test compounds dissolved in DMSO and PBS during 5 min at 37 °C. The final concentration of DMSO in the test medium and controls was 1%. After incubation cells were exposed to tert-butylhydroperoxide (1 nM) during 15 min to 37 °C under shaking. After treatment, lipid peroxidation was determined indirectly by the TBARs formation as described by De Azevedo et al. (2000). Results are expressed as percentage inhibition compared to controls (Mathiesen et al., 1995). Each determination was repeated four times. To calculate the IC_{50} values (concentration that produces a 50% inhibitory effect on the TBARs formation) the results were transformed to percentage of controls and the IC_{50} values were graphically obtained from the dose–response curves.

2.12. DPPH decoloration assay

The quenching of free radicals by extracts and purified fractions was evaluated spectrophotometrically at 517 nm by the decolouration of a methanol solution of DPPH according to Feresin et al. (2002). A freshly prepared DPPH solution (20 mg/L) was used for the assays. Samples were dissolved in methanol and the DPPH solution served as a control. The degree of decolouration indicates the free radical scavenging efficiency of the samples. Quercetin was used as reference free radical scavenger. The percentage of DPPH decolouration was calculated as follows:

$$decolouration\,(\%)$$
$$= 1 - \frac{(A\,compound\,with\,DPPH - A\,blank\,sample)}{A\,DPPH\,control} \times 100$$

Extracts were assayed at final concentrations of 200, 100, 50, 10 and 1 μg/mL. The IC_{50} was calculated when allowed according to the scavenging efficiency.

3. Results

Table 1 shows the results for the pharmacological in vivo assays of the extracts and purified fractions as well as

Table 1
Anti-inflammatory (AE), analgesic (AnE) and topic anti-inflammatory effect (TAE) induced by AA and TPA (TAE_{AA} and TAE_{TPA}, respectively) of *Buddleja globosa* extracts and reference drugs

Sample	Oral/topic dose (mg/kg)/(mg/ear)	% AE	% AnE	% TAE_{AA}	% TAE_{TPA}
Extract					
Global methanol	600/3	47.3 ± 2.1*	27.3 ± 2.6*	32.7 ± 7.9*	60.3 ± 7.9*
Hexane	600/3	17.2 ± 4.5	41.2 ± 5.1*	47.7 ± 8.0*	79.0 ± 8.0*
Dichloromethane defatted	600/3	43.7 ± 2.9*	76.7 ± 2.9*	64.4 ± 6.0*	83.8 ± 19.6*
Methanol-1	600/3	61.4 ± 1.8*	38.5 ± 3.2*	13.8 ± 13.5	56.7 ± 13.5*
Fraction					
CD4-N	300/1	n.t.	38.7 ± 5.0*	14.2 ± 1.2	78.2 ± 12.2*
CD5-N	300/1	n.t	23.1 ± 1.2*	0 ± 7.5	83.7 ± 6.5*
Reference drug					
Sodium naproxen	4	54.6 ± 0.8*			
	12.5		70 ± 1.8*		
Nimesulide	1			48.8 ± 3.9*	
Indomethacin	0.5				92.9 ± 3.2*

CD4-N, CD5-N: purified fractions from defatted dichloromethane extract; CD-4: steroid mixture, CD-5: β-sitosterol glucoside (main compound). Each value represents the mean ± S.E.M. of eight animals treated with samples or reference drugs. *$P \leq 0.05$.

Table 2
Antioxidant activity of *Buddleja globosa* extracts and fractions on xanthine oxidase (XO) and superoxide anion (SO) scavenging at 50 μg/mL, inhibition of lipid peroxidation (LPerox) at 200 μg/mL and bleaching of DPPH radical (IC$_{50}$, μg/mL)

Sample	% XO inh.	% SO inh.	% L Perox inh.	DPPH IC$_{50}$
Extracts				
Global methanol	0	51 ± 2.5*	60.0 ± 1.7*	8.4 ± 0.1*
Hexane	31.3 ± 1.5*	57 ± 3.5*	0	> >200
Dichloromethane defatted	11.1 ± 0.5*	0	65.0 ± 3.5*	25.3 ± 0.8*
Methanol-1	0	68.0 ± 3.0*	59.0 ± 2.0*	6.0 ± 0.4*
Fractions				
CD4-N	0	0	45.0 ± 2.6*	> >200
CD5-N	29.4 ± 1.5*	0	48.0 ± 2.9*	19.3 ± 0.4*
Reference drug				
Allopurinol (0.37 mM)	50.0 ± 0.5*			
Quercetin (0.17 mM)		72.0 ± 1.5*		1.07 ± 0.18*

Each value represents the mean value of three determinations ± standard deviation. *$P \leq 0.05$.

the reference compound sodium naproxen (SN) for *per os* anti-inflammatory and analgesic activities (Backhouse et al., 1997; Delporte et al., 2002). It also provides the dermal anti-inflammatory maximum effect of nimesulide (NM) and indomethacin (IND) (Delporte et al., 2005). Table 2 shows the antioxidant activity of *Buddleja globosa* extracts and fractions on xanthine oxidase (XO), superoxide anion (SO) scavenging at 50 μg/mL, inhibition of lipid peroxidation (LPerox) at 200 μg/mL and bleaching of DPPH radical (IC$_{50}$, μg/mL) together with the effect of the reference drugs allopurinol and quercetin.

The global methanol extract (GME) showed topic (higher in the TPA model) and *per os* anti-inflammatory effect, being less active as analgesic (Table 1). This complex extract contain triterpenoids, sterols, phenylethanoids and flavonoids and showed an important inhibition of lipid peroxidation (60%) as well as superoxide anion (SO) scavenging activity (51%) and bleaching of DPPH (IC$_{50}$: 8.4 μg/mL) but was inactive against the enzyme xanthine oxidase (Table 2).

The hexane extract (HE) containing the pentacyclic triterpenoids β- and α-amyrins (1.12%) showed mainly analgesic effect when administered *per os* while its anti-inflammatory topic effect was higher in the TPA-induced oedema (Table 1). The HE only showed superoxide anion scavenging effect (57% at 50 μg/mL) in the assay panel used for antioxidant activity (Table 2).

The defatted dichloromethane (DDCME) was the most active extract as analgesic, anti-inflammatory (by topic route) and antioxidant in the lipid peroxidation model (65% of inhibition), showing also free radical scavenging activity in DPPH assay, being inactive against SO inhibition (Table 2). Bioguided fractionation of this extract led to fractions CD4-N and CD5-N showing important effect, reducing topical inflammation induced by TPA by 78.2 and 83.7%, respectively (Table 1), with similar activity in lipid peroxidation inhibition, being CD5-N also active in the DPPH assay. The fraction CD4-N contained mixtures of β-amyrin and α-amyrin (3:2) (41 mg, 0.46%), β-sitosterol and stigmasterol as major components, together with campesterol, stigmastanol and ergosterol (175 mg, 1.95%), and β-sitosterol glucoside (92.7 mg, 1.03%). The frac-

tion CD5-N contained mainly β-sitosterol glucoside (362 mg, 1.78%). The DDCME extract contained triterpenoids (0.087%), steroids (0.37%) and β-sitosterol glucoside (0.97%) as active constituents.

From the methanol extract EMAT1 0.1% of β-sitosterol glucoside, 1.8% of verbascoside, 1.1% of luteolin 7-*O*-glucoside and 0.03% of apigenin 7-*O*-glucoside were isolated and purified for identification and pharmacological evaluations. It show *per os* anti-inflammatory and analgesic effect (61.4% and 38.5%, respectively) and was active as topic anti-inflammatory in TPA-induced oedema (56.7%) (Table 1). This polar extract was the most active as antioxidant in the SO, lipid peroxidation and DPPH assays, however, it was inactive against XO (Table 2).

4. Discussion and conclusion

A mixture of known triterpenes and steroids were isolated and identified from the active fractions of *Buddleja globosa*. The antinociceptive properties of a mixture of α- and β-amyrins have recently been demonstrated in several *in vivo* models. The mechanisms involved are independent of important endogenous analgesic systems, namely opioidergic, serotoninergic and noradrenergic, producing consistent peripheral, spinal and supraspinal antinociception in rodents, especially when assessed in inflammatory models of pain. Mechanisms are still not completely understood but the inhibition of protein kinase A- and protein kinase C- sensitive pathways might be involved (Otuki et al., 2005). A mixture of α-amyrin (43.7%), β-amyrin (24.9%) and bauerenol (31.4%) exhibited higher analgesic (51%) than anti-inflammatory (20%) activity (Villaseñor et al., 2004), similar to our findings for HE containing a mixture of both amyrins. Several triterpenoids, including α- and β-amyrin have been studied in different models for their anti-inflammatory effect and their structural requirements have been proposed (Recio et al., 1995). The effect of a similar mixture of α- and β-amyrin (2:1) has been related to a stabilizing action on mast cell membrane involved in inflammation process (Brinker et al., 2007).

Pharmacological properties reported in literature for steroids could explain the analgesic and anti-inflammatory effect found

in our study for the defatted dichloromethane extract (DDCME) and its fractions (CD4-N and CD5-N). A dose-dependent topical anti-inflammatory effect of stigmasterol and β-sitosterol in TPA model, also reducing myeloperoxydase activity (MPO), markedly influenced by neutrophil migration inhibition into inflamed tissue has been described (García et al., 1999). In addition, β-sitosterol proved to increase pain tolerance in both acetic acid-induced writhing and hot plate methods, widely used to evaluate analgesic activity (Villaseñor et al., 2002). β-Sitosterol and erythrodiol have shown a potent effect in the auricular oedema induced by TPA and AA assays and strongly inhibited the enzyme myeloperoxidase (De la Puerta et al., 2002). Furthermore, β-sitosterol has shown growth inhibition of human prostate cancer PC-3 cells, being effective in inducing apoptosis, and its mechanism may involve prostaglandin production (Awad et al., 2005). Ergosterol, another sterol present in "matico" is known to suppress LPS-induced inflammatory response (Kobori et al., 2007). All these information could explain the anti-inflammatory (*per os* and topic) and analgesic effects found for DDCME.

The anti-inflammatory (*per os* an topic in TPA-induced edema), analgesic and antioxidant activities found in the serial methanol extract (EMAT1), can be related to the verbascoside and luteolin 7-*O*-glucoside content of the sample. Pharmacological properties of flavonoids related to their antioxidant and anti-inflammatory activity have been reported (Kim et al., 2004; Feresin et al., 2002; Schmeda-Hirschmann et al., 2003; Lin et al., 2002). Inflammation and oxidative stress are associated with several diseases and flavonoids have been intensively investigated for anti-inflammatory and antioxidant properties (Middleton et al., 2000). The *in vitro* anti-inflammatory effect of luteolin 7-*O*-glucoside was assayed showing an inhibitory effect over induced human neutrophils (Jalil et al., 2003). It also presented an important inhibitory effect over matrix metalloproteinase-2 and -9 that may contribute to the modulating influence on extracellular matrix degradation and remodeling (Ende and Gerbhardt, 2004). Luteolin showed reduction of lipopolysaccharide (LPS)-induced COX-2 expression and completely suppressed PGE2 formation (Harris et al., 2006). Luteolin glucoside exhibited a moderate inhibition activity against both thromboxane and leukotriene synthesis and showed anti-inflammatory and antioxidant activities (Odontuya et al., 2005). It also inhibited the xanthine/xanthine oxidase-generated superoxide formation and reduced LPS-induced hydroxyl radical formation (Pereira et al., 2006). The protective effect of verbascoside against plasma lipid peroxidation during immobilization causing increasing free radical levels has been demonstrated. Its administration diminished erythrocyte membrane damage by reducing oxidative stress level, leading to a potential clinical application (Liu et al., 2003). The high redox potential as electron donor of verbascoside, protects cells against glucose oxidase-mediated cytotoxicity and apoptosis (Chiou et al., 2004), and a preventive potential may be attributed to the treatment of oxidative stress-mediated diseases (Kim et al., 2005). Verbascoside showed inhibitory effect on histamine and bradykinin-induced contractions and oral anti-inflammatory activity (Schapova et al., 1998), inhibited NO release in lipopolysaccharide-treated cells, effect related to the

expression of iNOS and its anti-inflammatory properties (Lee et al., 2005).

For the first time, the anti-inflammatory (topic and oral) and oral analgesic effect of the aerial parts of *Buddleja globosa* is reported. The free radical scavenging and antioxidant activities of total and sequential extracts were assessed and several metabolites were isolated and identified from the active fractions. The presence of these metabolites can explain, at least in part, the anti-inflammatory, analgesic and antioxidant activities of "matico" and support the traditional use of *Buddleja globosa* in Chilean traditional medicine.

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