Anti-inflammatory activity and phenolic profile of propolis from two locations in Región Metropolitana de Santiago, Chile

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ABSTRACT

Ethnopharmacological relevance: Propolis has long been used as a popular folk medicine due to its wide spectrum of alleged biological and pharmaceutical properties. In Chile, propolis is widely used by folklore medicine as an anti-inflammatory agent; however, this property has not been demonstrated by scientific methods.

Aim of the study: The objective of this study was to determine the anti-inflammatory activity in vivo and in vitro and to establish the phenolic profile of propolis collected in two localities in Región Metropolitana de Santiago (RM), Chile.

Materials and methods: Propolis was collected in the areas of Caleu and Buin, RM Chile. Following that, the samples were unwaxed to obtain the global ethanolic extracts of propolis (EEPs) and, from these, the serial extracts of dichloromethane (EEP-DCMs) and ethanol (EEP-EtOHs). The topic anti-inflammatory effect was evaluated through mouse ear edema induced by arachidonic acid (AA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) at a dose of 3 mg/ear. Nitric oxide (NO) measurements were determined spectrophotometrically (Greiss reagent) by the accumulation of nitrite in the medium of macrophages RAW 264.7 stimulated with the lipopolysaccharide (LPS, 1 μg/ml) for 24 h at different concentrations of the EEPs, EEP-DCMs and EEP-EtOHs (6.25–50.00 μg/mL). The content of total phenols and flavonoids were determined through the methods of Folin–Ciocalteau and AlCl3, respectively. The profile of phenolic compounds was determined by HPLC–UV–ESI-MS/MS.

Results: The EEP-EtOH (64%) and EEP (59%) of Buin were the most active in the inactivation induced by TPA and AA, respectively, being the anti-inflammatory effect stronger than the same Caleu extracts. Regarding the release of NO, all the extracts from the Buin propolis inhibited significantly its release in a concentration-dependent manner, this inhibition was stronger than the extracts from Caleu propolis.

Conclusions: Our research shows for the first time a comparative study of the topical in vivo activity of two Chilean propolis. Both propolis showed in vivo topical anti-inflammatory activity against AA and TPA, the most active was Buin propolis and this difference is due in part to the variations in total phenols and flavonoids content and the phenolic profile. The phenols and flavonoids content of Buin propolis was higher than Caleu propolis. The extracts from Buin propolis result in a lower release of NO.

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1. Introduction

Propolis is a resinous material elaborated by the bee Apis mellifera, n.v., through the recollection of the exudates from different plant species. The bees use propolis to repair combs, to strengthen the thin borders of the comb, and to make the entrance of the hive watertight or easier to defend. Propolis is also used as an embalming substance to cover the carcass of a hive invader which the bees have killed but cannot transport out of the hive. The bees cover the invader with propolis and wax, and the remains are left at the bottom or on one of the walls of the hive (Toreti et al., 2013). Propolis is constituted by resinous, sticky and balsamic substances; such as waxes, essential oils and pollen, among others (Tosi et al., 2006).
Folk medicine recommends the use of propolis, due to its anti-bacterial, anti-fungal, and anti-viral effects and its hepatoprotective and anti-inflammatory properties, to increase the resistance against infections and to treat gastroduodenal ulcers (Castaldo and Capasso, 2002). Propolis has attracted researchers’ interest in the last decades because of several biological and pharmacological properties, such as immunomodulatory, anti-tumor, anti-inflammatory, anti-oxidant, anti-bacterial, anti-fungal and anti-parasite, among others (Sfornic and Bankova, 2011). Besides, products containing propolis have been intensely marketed by the pharmaceutical industry and health-food stores (Sfornic, 2007). The ethnopharmacological approach, combined with chemical and biological methods, may provide useful pharmacological leads about the medical effects of propolis.

In Chilean propolis, only some of these properties have been evaluated, such as anti-oxidant (Astudillo et al., 2000; Castro et al., 2014; Russo et al., 2004), anti-bacterial (Saavedra et al., 2011), anti-fungal (Herrera et al., 2010) and anti-tumoral (Russo et al., 2004), among others. However, the anti-inflammatory activity through in vivo and in vitro methods has not been investigated.

The chemical composition of propolis is complex and varied, which is why a great amount of compounds have been identified, such as: alcohols, aldehydes, phenolic acids, amino acids, chalcones, flavonoids, lignans, triterpenes, steroids and sugars, among others. However, phenolic compounds are the most abundant (Righi et al., 2013).

In this research, we present a comparative study of the in vivo anti-inflammatory activity of two propolis recollected from the localities of Buin and Caleu, which have similar climate and soil conditions but whose main variation was in the surrounding vegetation. Different unwaxed extracts were prepared and their topical anti-inflammatory activities were evaluated in the edema model in mice ear, induced by 12-O-tetradecanoyl-phorbol-13-acetate (TPA) and arachidonic acid (AA). The total phenols and flavonoids content, phenolic profile and inhibitory effect of the release of nitric oxide (NO) were determined for each extract.

2. Materials and methods

2.1. Propolis samples

The samples were recollected during spring in 2011, the hives free of diseases were located in Buin (33°38’43.84”S; 70°39’59.69’’O) and Caleu (33°00’12.8”S; 70°59’37’’O), Región Metropolitana de Santiago (RM), Chile. The harvest was done with plastic meshes. Then, the meshes were preserved at −4 °C for 4 h; after this period, the meshes were flexed to release the propolis. Afterwards, the different samples were stored at −20 °C protected from the light.

Buin propolis presented a homogenous aspect, a rigid consistency with brown and green hues. Caleu propolis presented a heterogenous aspect, a rigid consistency, and green and yellow hues.

Buin and Caleu are known for having a Mediterranean weather, long dry season and rainy winter. It is a warm temperate weather in which winter rains are concentrated between May and August. The main difference between both locations is in the vegetation surrounding the hives, identified by the taxonomist Sebastian Teiller. In Buin, there were found: Agapanthus africanus (n.v. agapanto), Argyranthemum frutescens (n.v. paraquato), Cyperus alterniflorus (n.v. paragüita), Escallonia illitica (n.v. barraco), Lavandula angustifolia (n.v. lavanda), Medicago sativa (n.v. alfalfa), Nerium oleander (n.v. laurel de flor), Otholobium glandulosum (n.v. culén), Populus spp. (n.v. álamo), Salvia microphylla (salvia) and Verbena officinalis (n.v. verbena); and in Caleu: Santolina chamaecyparissus (n.v. manzanillera), Acacia caven (n.v. espino), Trifolium repens (n.v. trébol blanco), Cynodon dactylon (n.v. chépica), Plantago lanceolata (n.v. siete venas), Populus spp. (n.v. álamo) and Quillaja saponaria (n.v. quillay).

2.2. Preparation of propolis extracts

To elaborate the global ethanolic extracts of propolis (EEPs), initially a process of unwaxing was carried out in the crude propolis employing temperature cycles, according to the methodology proposed by Alencar et al. (2007) and Kalogeropoulos et al. (2009). 400 g of raw propolis from each locality was weighed, homogenized in a mortar and added to 750 ml of ethanol. Following this, the ethanolic mixture was introduced in a thermostated bath at 70 °C for 30 min; afterwards, it was cooled at room temperature and refrigerated at −20 °C for 12 h. Finally, it was filtered and the supernatant was preserved. This procedure was repeated 3 times with the aim of extracting the wax exhaustively. Once the propolis was unwaxed, the extracts were put in a rotary evaporator at 60 °C until the solvent was completely eliminated, obtaining the EEP.

The serial extracts were elaborated through successive extractions of the EEP with the solvents dichloromethane (DCM) and ethanol (EtOH), resulting in the extracts EEP-DCM y EEP-EtOH, respectively. Each extraction was carried out until the EEPs were completely exhausted; between each extraction the material was dried at room temperature before adding the new solvent. The serial extracts were concentrated in a rotary evaporator at reduced pressure.

2.3. In vivo topical anti-inflammatory activity

Two inflammatory agents, AA and TPA, were used to estimate the probable anti-inflammatory action mechanism of the propolis under study. The reference drugs used were indomethacin and nimesulide against TPA and AA, respectively.

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 bio-ethics norms of the Commission of the Instituto de Salud Pública de Chile (ISP) and the Facultad de Ciencias Químicas y Farmacéuticas of the Universidad de Chile (CBE2012-4).

Adult male CF-1 mice (20–25 g), obtained from the stock at the ISP, were used to assess the anti-inflammatory effect. All 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. For each of the samples under study, the anti-inflammatory activity was evaluated in two groups. One group of 8 treated mice and the other of 16 control mice. After 5 min of sample treatment (3 mg/ear of the EEP, EEP-DCM and EEP-EtOH), mice received 5 μg of TPA or 2 mg of AA (Sigma, St. Louis, MO, USA) as pro-inflammatory agents, dissolved in 20 μL of acetone (solvent does not interfere with the assay). Control subjects only received TPA or AA at the same concentration. Both, the sample and the TPA or AA, were applied to the inner (10 μL) and outer (10 μL) surfaces of the right ear. The left ear only received acetone. Mice were sacrificed by cervical dislocation (after 6 h of TPA and 1 h of AA), and a 6 mm diameter section of the right and left ears were cut and weighted. The weight differences between both ear sections correspond to the edema value. Topical anti-inflammatory effect (EA) was evaluated according to the following equation: \( \% EA = \left[ \frac{W_r - W_t}{W_r} \right] \times 100 \); where \( W_r \) and \( W_t \) are the difference median values of the weights of the right and the left ear sections of the control (\( W_r \)) and the treated animals (\( W_t \)) respectively (Delporte et al., 2003).
2.4. Inhibitory activity of NO release

2.4.1. Cell culture

The murine macrophage cell line RAW 264.7 was maintained in DMEM Ham’s F-12 medium supplemented with 10% fetal bovine serum, L-glutamine (1 mM), penicillin (100 U/mL), streptomycin (100 μg/mL), gentamicin (40 μg/mL), acquired in Life and Technology (Barcelona, Spain) and L-arginine (1 mM, Sigma, St. Louis, MO, USA) in a humidified 5% CO₂ atmosphere (Díaz-Vicedo et al., 2008; Girón et al., 2010).

2.4.2. MTT assay for cell viability

Cytotoxicity studies were assessed by the mitochondrial-dependent reduction of the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) to formazan. Macrophages were plated at a density of 3.25 × 10⁵ cells/well in 96-well plates. To determine the appropriate non-toxic concentrations for cells, cells were incubated in the presence of different concentrations of propolis samples (6.25–100.00 μg/mL) for 24 h, before they reacted with MTT (2 mg/mL in phosphate-buffered saline PBS, Sigma, St. Louis, MO, USA) at 37 °C for 1 h. The reaction product, formazan, was extracted with dimethyl sulphoxide (DMSO) and the absorbance was read at 550 nm. Assays were performed in triplicate, and results were expressed as the percent reduction in cell viability compared to untreated control cultures for at least three independent experiments (Cuadrado et al., 2012; Girón et al., 2010).

2.4.3. Inhibitory activity of NO release in RAW 264.7 macrophages

Macrophage cells were seeded in 96-well plates and were incubated with 1 μg/mL of lipopolysaccharide (LPS, Sigma, St. Louis, MO, USA) at 37 °C for 20 h in the presence of test samples or vehicle. The release of NO into phenol red-free medium was determined from the amount of accumulated nitrite, measured spectrophotometrically with the Griess reagent (Sigma, St. Louis, MO, USA). The compound N-(3-[Aminoemethyl]benzyl) acetamidine (1400W, Sigma, St. Louis, MO, USA) was used as positive control. The absorbance at 540 nm was compared to a NaNO₂ standard (Cuadrado et al., 2012; Girón et al., 2010).

2.5. Determination of total phenolic content in the propolis extracts

To determine the amount of total phenols present in the EEP, EEP-DCM and EEP-EtOH of each propolis, the method Folin–Ciochetteau was used according to what was suggested by Ciccó et al. (2009). 100 μL of hydroalcoholic solutions (methanol/water, 70:30 v/v) of the extracts (1 mg/mL) were mixed with 100 μL of the reactive Folin–Ciochetteau (Merck, Germany), after 2 min, 800 μL of Na₂CO₃ at 5% p/v were added. This mixture was heated in a bath at 40 °C for 20 min, then the absorbance was measured in a spectrophotometer Unicam UV–vis. The samples were evaluated in triplicate. The obtained absorbances were interpolated in a calibration curve (y = 0.1044x + 0.0097, R² = 0.9995, Fcalc = 0.3445 < Ftable 3.7083) of galic acid (2–7 μg/mL). The results were expressed as g equivalents of galic acid/100 g of dry extract of propolis.

2.6. Determination of flavonoids content in the propolis extracts

The AlCl₃ was employed to determine the content of flavonoids. Hydroalcoholic solutions (methanol/water, 70:30 v/v) were elaborated from the propolis extracts (1 mg/mL). Afterwards, 0.5 mL of the hydroalcoholic solutions of propolis were mixed with 0.5 mL of an AlCl₃ (0.5% p/v) solution, then it was brought to volume with methanol at 25 mL. The mixtures were left for 30 min the dark, then were placed in a quartz cuvette, and the absorbances at 465 nm were read in a spectrophotometer UV/vis Spectrometer Unicam UV3 (Popova et al., 2004). The results were interpolated in the calibration curve (y = 0.0087x – 0.002, R² = 0.9964, Fcalc = 0.7456 < Ftable 3.2592) previously constructed with quercetin (2–6 μg/mL). The results were expressed as g equivalent of quercetin/100 g of dry propolis extract.

2.7. Identification of propolis extracts by HPLC–UV–ESI-MS/MS

The propolis extracts were analyzed by HPLC Agilent 1100 (Agilent Technologies Inc., CA-USA) connected to the ion electrospray mass spectrometer Esquire 4000 ESI-IT (Bruker Daltonik GmbH, Germany). For the separation of HPLC, a column Symmetry C18 (Waters, MA, USA) 5 μm of 250 × 4.6 mm² was used. The separation of 20 μL of blank (methanol) and the propolis extracts (6000 ppm) was done at room temperature using a gradient system composed by the binary phases (A) formic acid 0.1% v/v in water and (B) formic acid 0.1% v/v in acetonitrile. The elution gradient was: 0–3 min 25% B, 3–10 min 30% B, 10–40 min 40% B, 40–60 min 60% B y 60–92 min 90% B. The flow employed was of 1.2 mL/min. The UV detection was made using a wave length of 290 nm. The ionization process (nebulization) using electrospray was done at 3.000 V assisted by nitrogen as the nebulizer gas at a temperature of 365 °C, pressure of 60 psi and flow of 10 L/min. The mass spectrometers were acquired in negative polarity. The analysis of the chromatograms and mass specters was done using the program Data Analysis 3.2 (Bruker Daltonik GmbH, Germany). The identification of the compounds was done revising the scientific literature (Castro et al., 2014; Falcão et al., 2010; Gardana et al., 2007; Medana et al., 2008; Pellati et al., 2011; Righi et al., 2013) and the data base MassBank. The relative amount of each compound in each extract was calculated. The molecular ion of each compound was selected by selective ion mode and then its area was integrated; the peak area of each compound of the EEPs was assigned as the 100% of abundance in order to calculate the relative percentage with their respective EEP-DCM and EEP-EtOH. With the aim of comparing the relative percentage between both propolis, the peak areas of the common compounds were compared. The 100% was assigned to the content of each compound in the Buin EEP and, therefore, the relative percentage obtained corresponded to the area of the peak of each compound from the Caleu extracts divided by the area of the Buin EEP multiplied by 100.

2.8. Statistical analysis

The significance of the results (p) of the in vivo assay was determined using Kruskall–Wallis test and Mann–Whitney test was used for the individual comparisons. One-way ANOVA and Tukey post-hoc multiple comparison tests were used to analyze data from the in vitro assay. The differences were considered significant for p ≤ 0.05.

3. Results and discussion

3.1. In vivo topical anti-inflammatory activity

The results are summarized in Table 1. All extracts of propolis showed topical anti-inflammatory activity. Generally, they were more active against TPA than AA. In the same way, against both anti-inflammatory agents, the extracts from Buin propolis were more active than the extracts from Caleu propolis.

The model of ear edema induction in mice, using different inflammatory agents (TPA and AA), is widely used to identify the probable topical anti-inflammatory effect of a substance under...
study and its probable action mechanism (Gábor, 2003). TPA induced inflammation develops more slowly than AA induced inflammation. TPA is a powerful tumor promoting agent found in croton oil (Croton tiglium L.). The topical administration of TPA provokes an acute edema with leukocyte infiltration, acting through the triggering of the protein kinase C (PKC), which is Ca<sup>2+</sup>- and phospholipids dependent. PKC plays an important role in the signal transduction of a great variety of substances that trigger cellular and proliferation functions. Active PKC acts at different levels, activating the nuclear factor kappa B (NF-κB), and reducing the immune response in T cells. They concluded that in concentrations between 6.25 and 50.00 μg/mL, Caleu propolis extracts were not cytotoxic and between 6.25 and 25.00 μg/mL, Buin propolis extracts were not cytotoxic (data not shown). Regarding the results of NO release, all the extracts of propolis inhibited significantly and in a dose-dependent way the production of this mediator. The Buin propolis extracts present higher values of inhibition: EEP, EEP-DCM and EEP-EtOH in a concentration of 25.00 μg/mL demonstrated an inhibition of NO release in a percentage of 73.2%, 55.3% and 67.8%, respectively. For their part, the EEP, EPP-DCM and EEP-EtOH of Caleu, in the same concentration, showed to inhibit the release of NO in a percentage of 35.5%, 45.4% and 31.7%, respectively. Therefore, the anti-inflammatory effect against TPA of said extracts could be in part linked to their capacity to inhibit of NOS-2. 

The positive control (1400W in a concentration of 12.50 μg/mL), which is a selective inhibitor of NOS-2, reached an inhibition of NO liberation of 90.0%.

In the bibliography, it is possible to find propolis from different origins that inhibit the liberation of NO, for example, Han et al. (2002), evaluated the release of NO in RAW 264.7 cells, activated by LPS and treated with a Korean EEP, determining that the maximum effect of inhibition was achieved at 100 μg/mL (78.9%). Song et al. (2002) determined that a Korean EEP at concentrations of 12.5, 25.0 and 50.0 μg/mL could significantly inhibit the liberation of NO in RAW 264.7 cells stimulated during 24 h with interferon gamma (IFN-γ). Subsequently, Blonska et al. (2004) determined that an EEP from Poland was able to inhibit in a 55.8% the liberation of NO at a concentration of 30 μg/mL in macrophages J74A.1 activated with LPS. They also proved that the main inhibitory component of the NO release were chrysin, galangin, kaempferol and quercetin. These components were all present in the propolis samples studied in this work (see Tables 2 and 3).
the propolis from Buin presented the highest content of phenolic compounds and flavonoids. EEP-OH was the extract with the most amount of total phenols and flavonoids. Adding to this, it is noteworthy that all the extracts from Buin propolis showed a higher anti-inflammatory activity in vivo and inhibition of NO release than the extracts from Caleu propolis.

The content of phenolic compounds in the different extracts obtained from the Buin and Caleu propolis are greater than the ones determined by Mohammadzadeh et al. (2007), who studied the flavonoids content in the propolis extracts from Buin and Caleu. However, the total phenol content of the propolis from Buin and Caleu are lower to the content of total phenols from propolis from the south of Chile (San Vicente de Tagua-Tagua), where results show a total phenol content of 63.9% (Russo et al., 2004).

3.4. Determination of flavonoids content in the propolis extracts

Regarding flavonoids content (Table 1), it should be noted that the method of evaluation of flavonoids, the formation of a colored complex with AlCl₃, allows the determination of flavones and flavonols (Popova et al., 2004). The Buin propolis presented, in all its extracts, a higher content of flavonoids regarding the extracts from Caleu propolis.

The flavonoids content of the propolis extracts from Buin were higher than those reported by Chang et al. (2002) who determined the content of flavonoids in six propolis samples: three samples from Taiwan, one from Brazil and two from China. The highest content was in one of the Chinese samples, 7.8%, and the lowest content corresponded to the Taiwanese sample, 2.3%. Socha et al. (2015) analyzed samples of propolis from various regions of Poland and found that the flavonoids content varied in 3.5–6.2%, these were significantly lower than the flavonoids content of the extracts elaborated with Buin propolis.

3.5. Identification of propolis extracts by HPLC–UV–ESI–MS/MS

Tables 2 and 3 present the phenolic profile of Caleu and Buin propolis, respectively. 12 common phenolic compounds were found between both propolis studied (quercetin, quercetin-3-methyl-ether, kaempferol, pinobanksin, quercetin-dimethyl-ether, luteolin-methyl-ether, drupanin, caffeic acid benzyl ester, pinocembrin, galangin, pinobanksin-3-O-acetate, and chrysin).

A semiquantification was done, using as reference Buin’s EEP (i.e., 100% was assigned to EEP of the Buin), of all the common compounds (data not shown). By comparing the relative percentages in some compounds present in the extracts obtained from Buin propolis, we can highlight that the contents of quercetin and kaempferol were higher in the EEP-OH, unlike caffeic acid benzyl ester, pinocembrin, galangin, pinobanksin-3-O-acetate and caffeic acid phenethyl ester (CAPE), which where predominant in Buin’s EEP-DCM. We relate to all extracts of the Caleu propolis, these obtained the minor relative percentages of the compounds found in both propolis.

In Chile, there are few studies referring to the chemical compositions of our propolis. Some of these investigations: (a) Muñoz et al. (2001), identified the compounds present in a propolis from the central zone of Chile (Colliguay, Región de Valparaíso), and found pinocembrin, galangin and kaempferol, among others, (b) Herrera et al. (2010), evaluated 6 commercial extracts of propolis obtained from the south zone of Chile (Temuco, IX Región de la Araucanía),
Table 3
HPLC–ESI–MS² (IT) data obtained for the analysis of propolis of Buin (EEP, EEP-DCM and EEP-EtOH) constituents in the negative ion mode.

<table>
<thead>
<tr>
<th>Compounds (EEP)</th>
<th>Tr (min)</th>
<th>M₀ (g/mol)</th>
<th>[M–H]⁻ (m/z)</th>
<th>Fragmentation MS² (m/z)</th>
<th>Semiquantification respect to EEP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>EEP-DCM %</td>
<td>EEP-EtOH %</td>
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<tr>
<td>1 Dihydroxyphenyl caffeate</td>
<td>2.1</td>
<td>282</td>
<td>281</td>
<td>134, 161, 178</td>
<td>69</td>
<td>228</td>
</tr>
<tr>
<td>2 Esculetin</td>
<td>2.2</td>
<td>178</td>
<td>177</td>
<td>133</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>3 7,8-Dihydroxy-6-methoxy coumarin</td>
<td>2.3</td>
<td>208</td>
<td>207</td>
<td>164, 191, 108</td>
<td>6</td>
<td>n.d</td>
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<tr>
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<td>3.3</td>
<td>282</td>
<td>281</td>
<td>134, 161, 178</td>
<td>103</td>
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<td>5 p-coumaric acid</td>
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<td>163</td>
<td>119</td>
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<td>79</td>
</tr>
<tr>
<td>6 Dicaffeoyl quinic acid</td>
<td>5.6</td>
<td>516</td>
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<td>353</td>
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<td>831</td>
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<tr>
<td>7 Esculetin</td>
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<tr>
<td>8 Quercetin</td>
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<tr>
<td>9 Pinobanksin-5-methyl-ether</td>
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<td>286</td>
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<td>315</td>
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<td>11 Apigenin</td>
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<td>328</td>
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<td>19 Drupanin</td>
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<td>20 Galangin-5-methyl-ether</td>
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<td>21 Quercetin-dimethyl-ether</td>
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<td>22 Caffeic acid prenyl ester</td>
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<td>25 Caffeic acid benzyl ester</td>
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<td>26 Pinocembrin</td>
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<td>27 Galangin</td>
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<td>28 Pinobanksin-5,7-dimethyl-ether</td>
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<td>29 Caffeic acid phenyl ethyl ester</td>
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<td>30 Pinobanksin-3-O-acetate</td>
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<td>33 Chrysin-5,7-dimethyl ether</td>
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<td>267, 165</td>
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<td>34 Pinobanksin-3-O-propionate</td>
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<td>35 Pinobanksin-3-O-butyrate</td>
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<td>37 Pinobanksin-3-O-hexanoate</td>
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<td>253, 271</td>
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n.d.: undetected; EEP global ethanolic extract of propolis; EEP-DCM serial dichloromethane extract of propolis; EEP-EtOH serial ethanolic extract of propolis. The semiquantification was carried out in respect to EEP and the relative percentages obtained correspond to the areas of the peaks of the EEP-DCM and EEP-EtOH extracts divided by the areas of EGE ≥ 100.

* Reference.

ab Data base.
Others compounds present in Buin and Caleu propolis, with anti-inflammatory effects were: caffeic acid benzyl ester, caffeic acid prenyl ester (Uwai et al., 2008), ferulic acid, p-coumaric acid (Fernández et al., 1998), apigenin, kaempferol, quercetin-7-methyl-ether (Kim et al., 2004), dicaffeoyl quinic acid (Peluso et al., 1995), caffeic acid cinnamyl ester (Nagaoka et al., 2003), pinocembrin (Rasul et al., 2013; Soromou et al., 2012) and vanillin (Makni et al., 2011).

It is conceivable that the observed action of propolis can be due, at least in part, to the content of CAPE and flavone derivatives, some of them: apigenin, chrysin, galangin, kaempferol and quercetin are frequently found in propolis (Blonska et al., 2004; Calixto et al., 2003; Kim et al., 2004; Nagaoka et al., 2003; Rossi et al., 2002), and are present in a higher relative percentage in the extracts obtained from Buin propolis than Caleu propolis and would contribute to the higher anti-inflammatory effect of Buin extracts in respect to the Caleu extracts. It should be noted that we found in Buin propolis, since it is known that the inflammatory process produces free radicals (Faroqui and Faroqui, 2010).

As it was previously pointed out, the localities of Buin and Caleu have similar climate and soil conditions and their main variation is the surrounding vegetation. Near the hives in Buin and Caleu were plant species of the genus Populus, the bud exudates of poplar trees and poplar type propolis also have similar qualitative compositions, but the quantitative compositions of the two propolis are different. (Díaz-Vieiedo, R., Hortelano, S., García, M.D., 2002). Unusual compounds. Anal. Bioanal. Chem. 396, 887–897.

4. Conclusions

Our results show the significant differences between the anti-inflammatory activity of the Buin and Caleu propolis, adding to the differences in their phenolic profile. Buin propolis exhibited a higher total phenol and flavonoids content regarding Caleu propolis, it was also the most active as a topical anti-inflammatory against the inflammatory agents TPA and AA, and was a more potent inhibitor of NO.

Although both propolis presented 12 common phenolic compounds, in Buin propolis they are present in a higher relative percentage, which could explain part of the higher anti-inflammatory effect of this propolis.

Our research show for the first time a comparative study of the anti-inflammatory topical activity in vivo of two Chilean propolis.


