**INTRODUCTION**

Species *Endopleura uchi* (Huber) Cuatrec. [sin. *Sacoglottis uchi* Huber] belongs to the Humiriaceae family and is typically wild in primary forests growing on non-flooded land, widely dispersed throughout the Amazon Basin. It is mainly found in the state of Amazonas, and popularly known as “uxi-amarelo”, “uxi-liso”, “uxi-pucu” and “pururu”.1,2 *Endopleura uchi* twigs and barks have been sold in open fairs, markets, nationwide online shops and commercial natural products outlets and used in the form of tea as a potent anti-inflammatory medicine against tumors, myomas, uterine infections, hepatoprotective,9 neuroprotective,10 anti-HIV11 and antifungal activities and bark extracts through RP-HPLC to find out the active principle bergenin quantification in different *Endopleura uchi* twigs and leaves extracts. Our findings showed the highest bergenin concentration in bark methanol extracts (4.75%) and the lowest concentration in twig aqueous extracts (1.89%). Phenolics quantification by Folin-Ciocalteu, revealed phenolic compounds level values from 16.69 to 43.02 mg GAE g⁻¹ dry extract. The ferric reducing antioxidant activity ranged from 230.43 to 567.89 mmol Fe²⁺ g⁻¹ dry extract. DPPH IC₅₀ free radicals showed to range from 12.04 to 24.20 µg mL⁻¹. Leaves' chloroform fraction exhibited the highest nitric oxide inhibiting activity bearing IC₅₀ = 3.2 µg mL⁻¹. Results show this species to hold significant bergenin concentrations as well as phenolic and anti-inflammatory compounds in all extracts suggesting it bear therapeutic potential.

**Phytochemical investigation of *Endopleura uchi* led to the isolation of a gallic acid derivate, known as bergenin (2) and friedelin (1), a pentacyclic triterpene. The present work also reports the bergenin quantification in different *Endopleura uchi* bark, twig and leaves extracts. Our findings showed the highest bergenin concentration in bark methanol extracts (4.75%) and the lowest concentration in twig aqueous extracts (1.89%). Phenolics quantification by Folin-Ciocalteu, revealed phenolic compounds level values from 16.69 to 43.02 mg GAE g⁻¹ dry extract. The ferric reducing antioxidant activity ranged from 230.43 to 567.89 mmol Fe²⁺ g⁻¹ dry extract. DPPH IC₅₀ free radicals showed to range from 12.04 to 24.20 µg mL⁻¹. Leaves' chloroform fraction exhibited the highest nitric oxide inhibiting activity bearing IC₅₀ = 3.2 µg mL⁻¹. Results show this species to hold significant bergenin concentrations as well as phenolic and anti-inflammatory compounds in all extracts suggesting it bear therapeutic potential.**

**Keywords:** Humiriaceae; liquid chromatography; anti-inflammatory agents; natural products; DPPH.
under the number 190992 were collected at Adolpho Ducke Reserve (INPA) located at Km 23 from Manaus (AM) during the dry period on October 11, 2010 and the rainy season on April 16, 2011. This project has been registered with the National Genetic Resource Management and Associated Traditional Knowledge Management System (SisGen – A04356A).

**General experimental procedures**

The NMR spectra were obtained on a Unity Inova spectrometer model 500 MHz (1H NMR) and 125 MHz (13C NMR) in CDCl₃ or CD₃OD (Cambridge Isotopes Laboratories Inc.). HR-ESI-MS and HR-APCI-MS analyses were measured in the positive ion mode (Bruker Daltonics, model II - TOF, APCI). Column chromatography was performed on silica LC60A (70 - 200 µm, Grace) and silica 60 (40 - 63 µm, Merck). Melting points (uncorrected) were recorded on a FISATOM 430 D Melting Point Apparatus.

**Extraction**

Leaves (F), twigs (G) and barks (C) were ground and dried. Aqueous extracts (EA) were prepared by decocion of 30 g of dried plant material in 500 mL boiling distilled water for 5 min as the popular use. Methanol extracts (EM) were prepared by soxhlet extraction, the solvent replaced every 6 hours, totaling 18 h of extraction. Ethanolic extract (EE) prepared by using 40 g of dried plant material in 400 mL of ethanol three times in ultrasound for 15 min each, followed by maceration for one day.

**Friedelin and bergenin isolation**

Friedelin was isolated from twigs and leaves hexane fractions. Bergenin was isolated from barks, twigs and leaves ethyl acetate fractions. Compounds were identified by 1H, 13C NMR, HOMOCOSY, HBC and MS spectrometry. Twigs (GEM-1, 9.6 g), leaves (FEM-1, 10.0 g) and barks (CEM-1, 13.8 g) methanol extracts were purified by chromatographic procedures. These crystals were purified by chromatographic column using silica gel LC60A (70-200 µm) and chloroform/ethanol (7:3) isocratic as the mobile phase to yield friedelin (1) (500.0 mg).

Friedelin (1): White crystalline solid; m.p: 252-255 °C; 1H δH (500 MHz, CDCl₃): 1.97 (m, H-1a), 1.69 (1H, dd, J = 5.0 and 13.0 Hz, H-1b), 2.39 (1H, dd, J = 7.5, 13.5 Hz, H-2b), 2.25 (1H, q, J = 6.5 Hz, H-4), 1.76 (1H, dd, J = 3.0, 16.0 Hz, H-6a), 1.28 (m, H-6b), 1.60 (m, H-7a), 1.48 (m, H-7b), 1.45 (m, H-8a), 1.40 (m, H-8b), 1.56 (m, H-10), 1.39 (m, H-11), 1.36 (m, H-12), 1.48 (m, H-15a), 1.28 (m, H-15b), 1.55 (m, H-16a), 1.37 (m, H-16b), 1.57 (m, H-18), 1.38 (m, H-19), 1.50 (m, H-21), 1.49 (m, H-22), 0.88 (3H, d, J = 6.5 Hz, H-23), 0.73 (3H, s, H-24), 0.89 (3H, s, H-25), 1.01 (3H, s, H-26), 1.05 (3H, s, H-27), 1.18 (3H, s, H-28), 1.00 (3H, s, H-29) and 0.96 (3H, s, H-30); 1C δC (125 MHz, CDCl₃): 22.6 (C-1), 41.8 (C-2), 213.5 (C-3), 58.5 (C-4), 42.4 (C-5), 41.6 (C-6), 18.5 (C-7), 53.6 (C-8), 37.8 (C-9), 59.8 (C-10), 35.9 (C-11), 30.8 (C-12), 40.0 (C-13), 38.6 (C-14), 33.1 (C-15), 36.3 (C-16), 30.3 (C-17), 43.1 (C-18), 35.7 (C-19), 28.5 (C-20), 32.7 (C-21), 39.6 (C-22), 7.1 (C-23), 15.0 (C-24), 18.2 (C-25), 20.6 (C-26), 19.0 (C-27), 32.4 (C-28), 32.1 (C-29) and 35.3 (C-30); HR-APCI-TOF-MS: 372.3942 [M + H]+, C₁₅H₂₃O₂; calc. 372.3934.

Bergenin (2): White crystalline solid; m.p: 150-152 °C; 1H δH (500 MHz, CDCl₃): 7.08 (1H, s, H-4), 4.95 (1H, d, J = 10 Hz, H-9), 3.69 (1H, m, H-11), 3.43 (1H, dd, J = 8.5, 8.5 Hz, H-12), 3.81 (1H, dd, J = 8.8, 9.5 Hz, H-13), 4.06 (1H, dd, J = 9.5, 10.0 Hz, H-14), 3.91 (3H, s, H-15), 3.68 (1H, m, H-16), 4.02 (1H, dd, J = 3.0, 13.0 Hz, H-16); 1C δC (125 MHz, CDCl₃): 164.6 (C-2), 118.2 (C-3), 109.9 (C-4), 151.1 (C-5), 141.6 (C-6), 148.2 (C-7), 116.1 (C-8), 73.1 (C-9), 81.9 (C-11), 70.7 (C-12), 74.4 (C-13), 80.2 (C-14), 59.8 (C-15), 73.1 (C-16), 73.1 (C-17), 73.1 (C-18), 73.1 (C-19), 73.1 (C-20), 73.1 (C-21), 73.1 (C-22), 73.1 (C-23), 73.1 (C-24), 73.1 (C-25), 73.1 (C-26), 73.1 (C-27), 73.1 (C-28), 73.1 (C-29) and 73.1 (C-30); HR-ESI-TOF-MS: 329.0866 [M + H]+, C₁₄H₁₀O₃; calc. 329.0867.

**Total phenolics determination**

The Folin-Ciocalteu method was used to quantify the total phenolic content in extracts and fractions. Two-hundred (200) µL of the sample (1 mg mL⁻¹ in methanol) were mixed with 1.5 mL of aqueous solution of Folin-Ciocalteu and Milli-Q water used as blank. The analyses were performed in triplicate and the total phenolic content mean was expressed in milligrams of gallic acid equivalents (GAE) per gram of dry extract. Gallic acid analytical curve was constructed in the range of 15.6-250 µg mL⁻¹ (y = 4.9604x – 0.0428, R² = 0.9986).

**DPPH free radical scavenging through antioxidant activity**

DPPH free radical scavenging capacity was measured by the photometric method described by Mensor. For each fraction, extract or standard (quercetin) six different dilutions were prepared (4-57 mg mL⁻¹ in methanol, 2.5 mL) and 1 mL of DPPH (0.2 mg mL⁻¹) added. After 30 min, in the dark at room temperature, a reading was taken at 518 nm in a spectrophotometer (Femto 800XI). Blank was obtained by the higher concentration samples and 2.5 mL methanol in 1 mL of DPPH as a negative control. The antioxidant activity was calculated as follows:

\[
AA\% = 100 - \left[ \frac{\text{Abs sample} - \text{Abs blank}}{\text{Abs control}} \right] \times 100 \tag{1}
\]
where, Abs blank = absorbance of the higher concentration sample, Abs control = absorbance of the negative control. The 50% inhibitory capacity (IC₅₀) was calculated from the slope with antioxidant activity versus sample concentration, which analyses were performed in triplicate.

### Antioxidant activity by FRAP method (ferric reducing antioxidant power)

The FRAP assay was performed as described by Luximon-Ramnam.²² The FRAP reagent was prepared fresh by mixing in 10 mM TPTZ (2,4,6-tripiridyl-triazine), 20 mM of iron (III) chloride solution and sodium acetate buffer (0.25 M, pH 3.5) at a ratio of 1:1:10. Then, 100 µL of the sample in methanol (1 mg mL⁻¹) were added to 300 µL of Milli-Q water and 3.0 mL of FRAP reagent. The absorbance was measured at 593 nm after being incubated for 4 minutes. Findings were expressed as Fe²⁺ µmol g⁻¹ dry weight of plant material and the analytical curve was constructed using FeSO₄ (62.5 – 2000 µmol L⁻¹; y = 0.0007x – 0.0428, R² = 0.9999).

### Cell culture and treatments

Murine macrophages J774 cells (donated from the cell bank of the Rio de Janeiro) were grown at 37 °C in an incubator containing 5% CO₂ in RPMI-1640 medium included fetal bovine serum (10%, FBS), streptomycin (50 µg mL⁻¹, Invitrogen) and penicillin (50 U mL⁻¹). The absorbance was measured at 593 nm after being incubated for 4 minutes. Findings were expressed as Fe²⁺ µmol g⁻¹ dry weight of plant material and the analytical curve was constructed using FeSO₄ (62.5 – 2000 µmol L⁻¹; y = 0.0007x – 0.0428, R² = 0.9999).

### NO production assay

After pre-incubation of cells (1x10⁶ cells mL⁻¹) with differently concentrated samples (2.5 to 25.0 µg mL⁻¹) for 2 h, cells were incubated for 24 hours either with or without LPS (1 mg mL⁻¹) at 37 °C in an incubator containing 5% CO₂. The same procedure was carried out for negative control and blank. Then, nitric oxide production (NO₃⁻) in the culture supernatant was determined using Griess reagent²³ in triplicate. Samples absorbance was measured at 560 nm using a microplate reader (DTX 800, Beckman). Sodium nitrite was used as a standard to calculate nitrite concentrations.

### Bergenin quantification

*Endopleura uchi* extracts bergenin quantification was performed using a method previously described by Nunomura *et al.*⁷ with some modifications. Analyses were performed by Liquid Chromatography using a SHIMADZU Prominence LC-20A, equipped by LC-10AT VP quaternary pump, automatic injector SIL-20A and DAD detector (DAD) SPD-M20A and data process using a LC-Solution software. The separation was performed in a Merck LiChrospher 100 RP-18e (250 mm x 4.0 mm, 5 µm and 120 A) column using, methanol as solvent B (20%) and THF solution in water (pH 2.0) as solvent A (80%), as mobile phase during 30 minutes. The mobile phase was degassed before analyses. The flow rate was 1.0 mL min⁻¹ and detection on 215, 254 and 272 nm. The column temperature was 25 °C and the injection volume 50 µL. The quantification was performed by integrating peaks using bergenin, as an external standard, diluted in methanol at 6.25, 12.5, 25.0, 50.0, 100.0 and 200.0 µg mL⁻¹ concentrations, analyzed intra-day and inter-day (R² > 0.995), in duplicate. Extracts were submitted to SPE using C18 cartridge (Sep-Pak) and analyzed at 250 µg mL⁻¹ in duplicate. Extract sample peaks identification was performed by comparison with bergenin by retention time and λmax in nm. Extract and standard samples were filtered on 0.2 µm Millipore membrane.

### RESULTS AND DISCUSSION

#### Bergenin quantification

Bergenin (2) was quantified in leaves, twigs and barks from *Endopleura uchi* by High Performance Liquid Chromatography-Diode Array Detector (HPLC-DAD) on aqueous and methanol extracts using Nunomura *et al.*⁷ method and some modifications. Bergenin standard solutions (6.25-200 µg mL⁻¹) were analyzed repeatedly intraday and interday and resulted in an excellent linear regression slope (R² > 0.995, CV < 5%) (Supplementary Material Table 1S and Figure 12S). Detection and quantification limits (DL) and (QL) showed a high detection level in addition to sensitivity for both bergenin quantification and trace analysis (Table 1).

Table 1. Curve data of standard solutions of bergenin (6.25-200 µg mL⁻¹)

<table>
<thead>
<tr>
<th>λ (nm)</th>
<th>Equation curve</th>
<th>Linear coefficient (R²)</th>
<th>DL⁻¹</th>
<th>QL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>272</td>
<td>y = 66.349,9399 x - 17.824,6393</td>
<td>1.000</td>
<td>9.85</td>
<td>29.84</td>
</tr>
<tr>
<td>254</td>
<td>y = 36.770,1945 x - 4.814,1791</td>
<td>1.000</td>
<td>8.88</td>
<td>26.92</td>
</tr>
<tr>
<td>215</td>
<td>y = 242.018,3217 x - 956.260,4163</td>
<td>0.996</td>
<td>0.78</td>
<td>2.37</td>
</tr>
</tbody>
</table>

Six points (n= 2, interday and intraday). *Detection limits (µg mL⁻¹).* Quantification limits (µg mL⁻¹).
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Table 2. Quantification of bergenin in different Endopleura uchi extracts with detection at 272 nm

<table>
<thead>
<tr>
<th>Extracta</th>
<th>Bergenin (%±CV)b</th>
<th>Extracta</th>
<th>Bergenin (%±CV)b</th>
<th>Extracta</th>
<th>Bergenin (%±CV)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA-1</td>
<td>3.31 ± 0.4</td>
<td>GEA-1</td>
<td>1.95 ± 1.0</td>
<td>FEA-1</td>
<td>3.24 ± 2.0</td>
</tr>
<tr>
<td>CEA-2</td>
<td>2.87 ± 3.0</td>
<td>GEA-2</td>
<td>1.89 ± 2.4</td>
<td>FEA-2</td>
<td>2.21 ± 2.1</td>
</tr>
<tr>
<td>CEM-1</td>
<td>4.17 ± 0.5</td>
<td>GEM-1</td>
<td>3.44 ± 5.0</td>
<td>FEM-1</td>
<td>2.23 ± 0.7</td>
</tr>
<tr>
<td>CEM-2</td>
<td>4.75 ± 1.0</td>
<td>GEM-2</td>
<td>2.66 ± 0.4</td>
<td>FEM-2</td>
<td>3.87 ± 2.0</td>
</tr>
</tbody>
</table>

a CEA = aqueous barks extract, CEM = methanol barks extract, GEA = aqueous twigs extract, GEM = methanol twigs extract, FEA = aqueous leaves extract, FEM = methanol leaves extract, 1 = rain season, 2 = dry season. b CV = Coefficient of Variation, from triplicate analyses (n=3).

Table 3. Total phenolics and antioxidant activities of extracts of Endopleura uchi

<table>
<thead>
<tr>
<th>Extracta</th>
<th>FT (mg GAEg⁻¹ dry extract)</th>
<th>FRAP (mmol Fe⁺²g⁻¹ dry extract)</th>
<th>DPPH (IC₅₀ µg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barks (value ± CV)c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEA-1</td>
<td>23.69 ± 3.7</td>
<td>230.43 ± 0.4</td>
<td>24.20 ± 1.6</td>
</tr>
<tr>
<td>CEA-2</td>
<td>23.94 ± 1.5</td>
<td>239.30 ± 0.8</td>
<td>22.13 ± 0.4</td>
</tr>
<tr>
<td>CEM-1</td>
<td>42.49 ± 0.7</td>
<td>449.47 ± 0.3</td>
<td>15.63 ± 2.6</td>
</tr>
<tr>
<td>CEM-2</td>
<td>42.17 ± 0.1</td>
<td>495.55 ± 0.9</td>
<td>12.04 ± 0.7</td>
</tr>
<tr>
<td>CEE-1</td>
<td>18.43 ± 2.4</td>
<td>241.93 ± 0.6</td>
<td>13.89 ± 0.3</td>
</tr>
<tr>
<td>CEE-2</td>
<td>26.49 ± 1.0</td>
<td>326.53 ± 0.7</td>
<td>12.51 ± 2.0</td>
</tr>
<tr>
<td>Twigs (value ± CV)c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GEA-1</td>
<td>27.94 ± 1.4</td>
<td>359.27 ± 0.2</td>
<td>13.89 ± 4.2</td>
</tr>
<tr>
<td>GEA-2</td>
<td>26.04 ± 4.3</td>
<td>316.97 ± 1.4</td>
<td>12.36 ± 1.4</td>
</tr>
<tr>
<td>GEM-1</td>
<td>42.16 ± 2.8</td>
<td>527.85 ± 0.8</td>
<td>13.62 ± 0.9</td>
</tr>
<tr>
<td>GEM-2</td>
<td>40.03 ± 1.3</td>
<td>512.58 ± 1.2</td>
<td>12.08 ± 2.3</td>
</tr>
<tr>
<td>GEE-1</td>
<td>16.69 ± 1.3</td>
<td>211.47 ± 1.6</td>
<td>13.51 ± 1.7</td>
</tr>
<tr>
<td>GEE-2</td>
<td>21.68 ± 0.5</td>
<td>290.24 ± 1.0</td>
<td>12.38 ± 1.7</td>
</tr>
<tr>
<td>Leaves (value ± CV)c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEA-1</td>
<td>38.94 ± 2.0</td>
<td>567.89 ± 2.7</td>
<td>14.76 ± 1.1</td>
</tr>
<tr>
<td>FEA-2</td>
<td>35.86 ± 1.6</td>
<td>469.99 ± 0.6</td>
<td>18.40 ± 0.9</td>
</tr>
<tr>
<td>FEM-1</td>
<td>43.02 ± 2.6</td>
<td>512.39 ± 0.9</td>
<td>14.00 ± 1.7</td>
</tr>
<tr>
<td>FEM-2</td>
<td>38.24 ± 3.1</td>
<td>476.35 ± 1.8</td>
<td>20.36 ± 0.3</td>
</tr>
<tr>
<td>FEE-1</td>
<td>32.46 ± 2.9</td>
<td>395.96 ± 0.4</td>
<td>17.50 ± 0.3</td>
</tr>
<tr>
<td>FEE-2</td>
<td>28.30 ± 1.4</td>
<td>365.38 ± 0.5</td>
<td>20.60 ± 1.5</td>
</tr>
<tr>
<td>Standard (value ± CV)c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>-</td>
<td>-</td>
<td>4.50 ± 0.4</td>
</tr>
<tr>
<td>Bergenin</td>
<td>-</td>
<td>-</td>
<td>&gt;1000</td>
</tr>
</tbody>
</table>

a CEA = aqueous barks extract, CEM = methanol barks extract, GEE = ethanol twigs extract, GEA = aqueous twigs extract, GEM = methanol twigs extract, GEE = ethanol twigs extract, FEA = aqueous leaves extract, FEM = methanol leaves extract, FEE = ethanolextract of leaves, 1 = rain season, 2 = dry season. b GAE = gallic acid equivalent. c Coefficient of Variation (CV), from triplicate analyses (n=3).

The antioxidant capacity was accomplished through two different methods as a way of attaining better results and avoiding unreal conclusions due to each method’s limitations.26,27 The aqueous, ethanol and methanol extracts were tested by FRAP (Ferric Reduction Power Assay) and by DPPH free radical scavenging methods according to Table 3.

Twigs, leaves and barks methanol extracts, respectively, resulted in the highest antioxidant activities by FRAP method. These extracts also presented the best phenolic compounds concentration and, these findings suggest these compounds be responsible for the antioxidant activity achieved by FRAP. In aqueous extracts the antioxidant activity was higher in leaves (469.99-567.89 mmol Fe⁺² g⁻¹) and twigs (316.97-359.27 mmol Fe⁺² g⁻¹) and barks (230.43-239.30 mmol Fe⁺² g⁻¹), respectively. Leaves ethanol extracts presented better results (365.38-395.96 mmol Fe⁺² g⁻¹) than barks (241.93-326.53 mmol Fe⁺² g⁻¹) and twigs (211.47-290.24 mmol Fe⁺² g⁻¹) ones. Bergenin presented lower values (176.1 mmol Fe⁺² g⁻¹) than Endopleura uchi extracts by FRAP method.

DPPH free radical scavenging capacity by the method is a rapid method to assess the compounds and extracts antioxidant activity.26 Our findings were compared with quercetin as a positive control (IC₅₀ = 4.50 µg mL⁻¹).

Extracts from twigs resulted in the best activities (IC₅₀ = 12.08-13.89 µg mL⁻¹), followed by leaves (IC₅₀ = 14.00-20.60 µg mL⁻¹) and barks (IC₅₀ = 12.04 to 24.20 µg mL⁻¹).

Although bergenin is the main extracts component, it presented a low antioxidant activity by DPPH method. The concentration of 1000 µg mL⁻¹ inhibits only 33% of DPPH radical scavenging activity, therefore the concentration to inhibit 50% should be higher than 1000 µg mL⁻¹ that is considered no significant antioxidant activity. According to Subramanian et al.,26 bergenin showed mild activity by the methods of DPPH, FRAP and nitric oxide inhibition. The antioxidant activity of extracts by DPPH method suggests Endopleura uchi extracts’ potential antioxidant activity when compared with the leaves’ commercial extracts from Ginkgo biloba (IC₅₀ = 40 µg mL⁻¹)
and other Brazilian plants used as antioxidants.\textsuperscript{25} The hydroxyl groups from phenolic compounds as flavonoids, phenolic acids, tannins, and other compounds can be responsible for these antioxidant activities.\textsuperscript{31-33}

LPS-induced NO production inhibition

According to traditional medicine, the species \textit{Endopleura uchi} is mainly used for the treatment of menstrual disorders and uterine inflammation, therefore its effect on the aqueous extracts, chloroform and ethyl acetate fractions inflammatory response through production of inflammatory mediators by LPS-induced in murine macrophage cells (J774), has been ascertained. It measured the ability to inhibit inflammatory mediators NO production.\textsuperscript{25} Macrophage cells were exposed to LPS for NO-stimulation and treated with extracts and fractions in the concentration of 25 µg mL\textsuperscript{-1}. The tested samples showed cytotoxicity at concentrations above 100 µg mL\textsuperscript{-1}, so 25 µg mL\textsuperscript{-1} was considered safe to test the samples. The negative control (cells + LPS) produced 8.5 µm of NO and the positive control (PC) (cells) produced 0.1 µm of the inflammatory mediator. The results showed cells treated with the extracts and fractions of \textit{Endopleura uchi} to produce a significant inhibition (Figure 2). The best results were observed in leaves chloroform fractions (FEMC) and chloroform (FEMC) and bark chloroform fraction (CEMC) resulting in 0.1, 0.5, 0.6 and 0.6 µm of NO respectively in LPS-induced NO production. The other extracts and fractions obtained NO production between 1.4-3.5 µm, below the negative control (8.5 µm of NO). When assessing the inhibitory capacity one finds the chloroform fractions of the leaves (FEMC) and bark (CEMC) de \textit{Endopleura uchi} to be the most active in producing IC\textsubscript{50} = 3.2 and 4.8 µg mL\textsuperscript{-1} respectively. The remaining extracts and fractions showed values between IC\textsubscript{50} = 7-12 µg mL\textsuperscript{-1} (Figure 3).

![Figure 2](image)

**Figure 2.** Effect of concentration 25 µg mL\textsuperscript{-1} of the extracts and fractions of \textit{Endopleura uchi} in the production of NO in culture of macrophages stimulated by LPS. Values are expressed as mean ± standard deviation obtained from analyzes in triplicate (n=3). NC = negative control; PC = positive control; CEA = aqueous barks extract; CEMC = barks chloroform fraction; GEA = aqueous twigs extract; GEMAc = twigs ethyl acetate fraction; GEMC = twigs chloroform fraction; FEA = aqueous leaves extract; FEMAc = leaves ethyl acetate fraction; FEMC = leaves chloroform fraction

Studies show evidence that the NO molecule, when produced in excess, can contribute to certain pathological conditions such as rheumatoid arthritis,\textsuperscript{34} Alzheimer’s and Parkinson’s\textsuperscript{35,36} diseases among others, therefore there is growing demand for substances capable of attenuating the NO production, which may have estimable therapeutic value in the treatment of excess nitric oxide-induced pathophysiological conditions.\textsuperscript{36} The results indicate the extracts and fractions of \textit{Endopleura uchi} to show NO inhibitory effect, therefore, it can be regarded as sources of active substances in the treatment of inflammatory processes. These findings should be taken into account for the isolation and identification of these active substances.

**CONCLUSIONS**

Since the findings showed to be positive for antioxidant activity by iron reduction, DPPH radical scavenging capacity methods, as well as an anti-inflammatory activity through NO suppression, they suggest that species \textit{Endopleura uchi} can be considered an important plant material bearing therapeutic potential. Quantification showed a significant amount of bergenin in its leaves and twigs, indicating that the substance bearing different biological activities may be attained from other plant parts apart from the barks. These findings can serve as the basis for preparing antioxidant and anti-inflammatory compounds that may contribute to the safer use of this plant species along with the development of possible herbal products.

**SUPPLEMENTARY MATERIAL**

Figures related to the results of some experiments used in this article are in the supplementary material that is available in http://quimicanova.sbq.org.br in the form of an open PDF file.

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