Bioprospection for the Sustainable Use of Wetlands: A Case Study from the Pantanal

Claudia L. Strada, ELiana F.C. Dores, E.L. Dall’Oglio, V.C.Silva and Paulo Teixeira de Sousa Jr*

9th INTECOL
International Wetlands Conference
June 3-8, 2012
Orlando, Fl - USA
THE PANTANAL

- The largest tropical wetland;
- Rich biological and cultural diversity;
  865 birds species; 263 fish species;
  1860 angiosperms species; etc...
- Cultural contribution from Amerindian, African and European population

- HOWEVER: Economic development in highlands – significant impacts;
  Urgent action to mitigate.
A rich tradition in the use of Medicinal Plants;

e.g. *Echinodorus macrophylus*

Its leaves are used as Infusion for treating:

Skin and venereal diseases, arthritis, rheumatism, as a diuretic, blood cleanser, anti-inflammatory, antihypertensive, liver disease.

BRANDÃO *et al.*, 2009
Echinodorus Genus

- Widespread in the Americas;
- Largest Alismataceae genus;
- 45 species;
- 17 in Brazil.

Folk use:
- Diuretic, anti-inflammatory, anti-rheumatic, skin care, among others;

Chemistry: flavonoids, acids and terpenoids.

GARCIA, 2010; SCHNITZLER et al., 2007; SHIGEMORI et al., 2002; COSTA, 2006; BEVILAQUA, 2001
**Echinodorus macrophyllus**

- Aquatic plant;
- Grows in riverbanks and marshy lowlands.
- Difficult identification;
- 70 cm long peciole;

MATIAS, 2010; LORENZI & MATOS, 2002; Leite, 2007
**Echinodorus macrophyllus**

- Present in South America, mainly in Brazil.

- Known as: Chapéu de couro, chá mineiro, erva de pântano and erva de brejo.

- Distributed in wetlands:
  - Caatinga
  - Cerrado
  - Mata Atlântica

- Regions:
  - North: RR
  - Northeast: PI, BA
  - Midwest: MT, MS
  - Southeast: RJ, SP, MG
  - South: PR

MATIAS, 2010; LORENZI & MATOS, 2002
Chemical-pharmacological studies: *E. grandiflorus e macrophyllus*;

Main constituents: Terpenes (diterpenes) and flavonoids

**Key word:** *Echinodorus*
E. Grandiflorus - Literature background

5 Flavones

- Swertisine - $R_1=\text{MeO}; R_2=\text{H}; R_3=\text{OH}$
- Isovitexine - $R_1=R_3=\text{OH}; R_2=\text{H}$
- Swertiajaponine - $R_1=\text{MeO}; R_2=R_3=\text{OH}$
- Isoorientine - $R_1=R_2=R_3=\text{OH}$
- Isoorientine-7,3'-dimetil éter - $R_1=\text{MeO}; R_2=\text{MeO}; R_3=\text{OH}$

Trans-aconitic acid

Chiconic acid
Echinodorus macrophyllus - Literature Background

4 Flavones

- Vitexin: $R_1 = R_3 = H; R_2 = \text{Gly}$
- Isovitexin: $R_1 = \text{Gly}; R_2 = R_3 = H$
- Orientin: $R_1 = H; R_2 = \text{Gly}; R_3 = \text{OH}$
- Isoorientin: $R_1 = \text{Gly}; R_2 = H; R_3 = \text{OH}$

Fenolic acid

Ferulic acid

TANUS-RANGEL et al., 2010; COSENZA et al., 2010; Prabhakar et al., 1981
Echinodorus macrophyllus - Literature Background

6 Nitrogen-containing Clerodane Diterpenoids

ECHINOFILINS

A: Y = H, H; X = O; R = \(-\text{C}_2\text{H}_4\text{-PhOH}\)

B: Y = H, H; X = O; R = \(-\text{C}_4\text{H}_8\text{N-arylOCH}_3\)

C: Y = H, H; X = O; R = H

D: Y = O; X = H, H; R = \(-\text{C}_2\text{H}_4\text{-PhOH}\)

E: Y = O; X = H, H; R = \(-\text{C}_4\text{H}_8\text{N-arylOCH}_3\)

F: Y = O; X = H, H; R = H
Echinodorus macrophyllus - Literature Background

2 Cembrane-like diterpenoids

A: R = H; R₁ = OH
B: R = OH; R₁ = H

Echinolides

B: R = OH; dihidro 5,6
C: R = H; Δ₇,₉

3 Labdane-like diterpenoids

Chapecoderins
Echinodorus macrophyllus - Literature Background

- EtOH-H₂O (7:3) extract caused inhibition in rat paw edema (anti-inflammatory activity);

![Chemical structures of Isovitexin and Vitexin]

TANUS-RANGEL et al., 2010
Objectives

General

• To develop an HPLC-based method for quality control of possible anti-inflammatory phytomedicines produced from *E. macrophyllus* leaves;
Objectives

Specific

- To find suitable potential chemical markers from the EtOH-H$_2$O (7:3) extracts of *E. macrophyllus* leaves;
- To carry out quantitative HPLC-DAAD analyses of the potential chemical markers in order to study their spatial variation;
- To verify a possible correlation between the potential chemical markers concentration and the anti-inflammatory activity.
Choice of Chemical Markers

**Flavonoids x Diterpenoids**

**Flavonoids:**

- Commercially available;
- Chemically stable;
- Present in relatively high concentration in the plant;
- Easily detectable by ultraviolet detectors;
- Described in the literature as anti-inflammatory;
### Experimental: Collection sites

#### Table 1 – Sites of collection of *E. macrophyllus*

<table>
<thead>
<tr>
<th>Code</th>
<th>Locality</th>
<th>Coordinates</th>
<th>Voucher specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>Juína-MT</td>
<td>15°56'02,37&quot;S; 56°36'08,97&quot;W</td>
<td>31645</td>
</tr>
<tr>
<td>L2</td>
<td>Poconé-MT</td>
<td>16°31'57,40&quot;S; 56°43'53,00&quot;W</td>
<td>33635</td>
</tr>
<tr>
<td>L3</td>
<td>Chapada dos Guimarães-MT</td>
<td>15°36'02,30&quot;S; 56°03'44,00&quot;W</td>
<td>33637</td>
</tr>
<tr>
<td>L4</td>
<td>Dom Aquino-MT</td>
<td>15°48'20,40&quot;S; 54°55'00,20&quot;W</td>
<td>33638</td>
</tr>
<tr>
<td>L5</td>
<td>Cuiabá-MT</td>
<td>15°42'03,80&quot;S; 55°53'11,20&quot;W</td>
<td>33639</td>
</tr>
<tr>
<td>L6</td>
<td>Market place-Chapada dos Guimarães</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>L7</td>
<td>Campo Grande -MS</td>
<td>20°30'25,79&quot;S; 54°34'24,80&quot;W</td>
<td>33665</td>
</tr>
<tr>
<td>L8</td>
<td>Campo Grande -MS</td>
<td>20°30'25,79&quot;S; 54°34'24,87&quot;W</td>
<td>33665</td>
</tr>
<tr>
<td>L9</td>
<td>Campo Grande -MS</td>
<td>20°28'41,28&quot;S; 54°34'00,06&quot;W</td>
<td>33665</td>
</tr>
<tr>
<td>L10</td>
<td>Campo Grande -MS</td>
<td>20°29'38,84&quot;S; 54°35'02,52&quot;W</td>
<td>33665</td>
</tr>
<tr>
<td>L11</td>
<td>Campo Grande -MS</td>
<td>20°30’04,02&quot;S; 54°36’05,52&quot;W</td>
<td>33665</td>
</tr>
</tbody>
</table>
## Experimental: HPLC Method Development

### Table 2 – Experimental HPLC-DAAD conditions

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Varian Pro Star 5.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump</td>
<td>quaternary model 240</td>
</tr>
<tr>
<td>injector</td>
<td>automatic model 410</td>
</tr>
<tr>
<td>detector</td>
<td>absorbance UV-DAD model 330 e</td>
</tr>
<tr>
<td>Estationary phase</td>
<td>C18 (250 x 4.6 mm D.I., 5 μm; HiChrom)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>CH₃OH; 0.05% aq. TFA and CH₃CN (gradient)</td>
</tr>
<tr>
<td>Stabilization time</td>
<td>2 min</td>
</tr>
<tr>
<td>Mobile phase flow</td>
<td>1 μL min⁻¹</td>
</tr>
<tr>
<td>Injection volume</td>
<td>4 μL</td>
</tr>
<tr>
<td>Wavelength</td>
<td>270 nm</td>
</tr>
<tr>
<td>Internal standard</td>
<td>Catechin</td>
</tr>
</tbody>
</table>
Experimental:
Method optimization

Table 3: Elution gradient

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%MeCN</th>
<th>%Aq. TFA (0.05%)</th>
<th>%MeOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>75</td>
<td>15</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>75</td>
<td>15</td>
</tr>
<tr>
<td>20</td>
<td>15</td>
<td>65</td>
<td>20</td>
</tr>
<tr>
<td>25</td>
<td>20</td>
<td>55</td>
<td>25</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>75</td>
<td>15</td>
</tr>
</tbody>
</table>
Results and discussion

Method optimization

Resolution:

Vit/Vitexin-2-O-rhamnoside: 0.62
Vitexin/Isovitexin: 1.16

Figure 1: Chromatogram – Standards Catechin (IS), Vitexin-2-O-rhamnoside, vitexin and isovitexin.

Figure 2: UV from IS (catechin).

Figure 3: UV from Vitexin-2-O-rhamnoside, vitexin and isovitexin.
Results and discussion

Identification of Vitexin in EmE

Figure 4:
5a) chromatogram of EmE (from site 1 – L1);
5b) chromatogram of EmE (L1) co-injected with vitexin [0.8 μg.mL⁻¹]
Results and discussion

Identification of Vitexin-2-O-rhamnoside

Figure 5:
6a) chromatogram of EmE (from site 6 - L6);
6b) chromatogram of EmE (L6) co-injected with vitexin-2-O-rhamnoside [0.8 µg.mL⁻¹].
Results and discussion

Identification of Isovitexin

**Figure 6:**
7a) chromatogram of EmE (from site 4- L4);
7b) chromatogram of EmE (L4) co-injected with isovitexin [0.8 μg.mL⁻¹].
Results and discussion: Calibration curves

Figure 8: Vitexin-2-O-rhaminoside calibration curve

- For 0.5-8.0 µG.mL⁻¹:
  \[ y = 0.1025x + 0.0456 \]
  \[ R^2 = 0.99934 \]

- For 8.0-20.0 µG.mL⁻¹:
  \[ y = 0.877x + 0.1651 \]
  \[ R^2 = 0.9999 \]
Results and discussion:

Calibration curves

Figure 9: Vitexin calibration curve

- For 0.5-8.0 µG.mL\(^{-1}\):
  \[ y = 0.2242x + 0.0136 \]
  \[ R^2 = 0.9993 \]

- For 8.0-20.0 µG.mL\(^{-1}\):
  \[ y = 0.1562x + 0.574 \]
  \[ R^2 = 0.9999 \]
Results and discussion:

Calibration curves

**Figure 10: Isovitexin calibration curve**

\[ y = 0.2512x + 0.0072 \quad R^2 = 0.9995 \]

0.5-8.0 µG.mL\(^{-1}\)

\[ y = 0.2073x + 0.3473 \quad R^2 = 0.9996 \]

8.0-20.0 µG.mL\(^{-1}\)
Results and discussion

Quantification of vitexin, vitexin-2-O-rhamnoside e isovitexin.

Table 5:

<table>
<thead>
<tr>
<th>Site</th>
<th>Vitexin-2-O-rhamnoside (µg/g)</th>
<th>Vitexin (µg/g)</th>
<th>Isovitexin (µG/G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Not detected</td>
<td>0.004</td>
<td>0.024</td>
</tr>
<tr>
<td>2</td>
<td>13.460</td>
<td>Not detected</td>
<td>0.720</td>
</tr>
<tr>
<td>3</td>
<td>Not detected</td>
<td>Not detected</td>
<td>1.060</td>
</tr>
<tr>
<td>4</td>
<td>0.128</td>
<td>Not detected</td>
<td>0.620</td>
</tr>
<tr>
<td>5</td>
<td>5.430</td>
<td>Not detected</td>
<td>0.470</td>
</tr>
<tr>
<td>6</td>
<td>33.130</td>
<td>Not detected</td>
<td>2.440</td>
</tr>
<tr>
<td>7</td>
<td>0.036</td>
<td>Not detected</td>
<td>6.750</td>
</tr>
<tr>
<td>8</td>
<td>0.089</td>
<td>Not detected</td>
<td>14.68</td>
</tr>
<tr>
<td>9</td>
<td>0.028</td>
<td>Not detected</td>
<td>9.220</td>
</tr>
<tr>
<td>10</td>
<td>0.220</td>
<td>Not detected</td>
<td>6.990</td>
</tr>
<tr>
<td>11</td>
<td>0.051</td>
<td>Not detected</td>
<td>5.710</td>
</tr>
</tbody>
</table>
Conclusions

• The chromatographic profile of samples collected at 11 different sites showed large differences in the concentrations of the chemical markers, except for vitexin, which was detected in only 1 site;

• Isovitexin was the only flavonoid found in all sites, presenting higher concentrations in sites 8-10 (Pantanal from MS);

• Vitexin-2-O-rhaminoside at higher concentrations was detected in samples from sites 2, 5 and 6 (market place), collected in the Pantanal from MT;

• To the best of our knowledge, vitexin-2-O-rhaminoside was detected for the first time in *E. macrophylus*;

• Preliminary pharmacological assays did not show a concentration x activity correlation; therefore, the flavonoids seem not to be have been a good choice as chemical markers.
Thank you!
Visit us
pauloteixeiradesousa@gmail.com
References


