DETERMINATION OF β-ECDYSONE IN INFUSIONS OF DIFFERENT ORGANS OF BRAZILIAN GINSENG (PFAFFIA GLOMERATA) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract: Pfaffia glomerata (Spreng.) Pedersen is a medicinal plant commonly known as “For everything” and Brazilian ginseng. Currently, only the roots have commercial value, as they are known to contain β-ecdysone. The roots of Pfaffia glomerata are used in Brazilian folk medicine, especially as tonics, aphrodisiacs and antidiabetics. Also, based on ethnobotanical data, such as roots and leaves of the plant previously used as general stimulants, tranquillizers, anti-rheumatic, anti-inflammatory, anti-inflammatory, febrifuges, internal and external healing, hemorrhoidal, in vision and memory, for the treatment of gastric disorders, arthritis, arthrosis, anemia, illness and pain. This study aimed to determine the concentration of β-ecdysone in aqueous infusion of leaves, flowers, roots and stems of 6-month-old and 3-year-old P. glomerata individuals by high-performance liquid chromatography. The leaves had the highest concentrations of β-ecdysone in both ages (1.65 ± 0.14% for the three-year-old individual and 1.06 ± 0.10% for the six-month-old individual). The sample of flowers, root and stem of the three-year-old showed: 1.14 ± 0.10%, 0.68 ± 0.05% and 0.62 ± 0.06% β-ecdysone, respectively. In the six-month-old individual, levels of 0.57 ± 0.02%, 0.51 ± 0.12% and 0.43 ± 0.12% were observed for flowers, root and stem, respectively. The results obtained that the aerial parts of the plant can also be of commercial interest, since the leaves presented the highest concentrations than another parts of the plant at both ages.

Keywords: β-Ecdysone. Pfaffia glomerata. High-performance liquid chromatography. Brazilian ginseng.

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Resumo: *Pfaffia glomerata* (Spreng.) Pedersen é uma planta medicinal conhecida como “Paratudo” e ginseng brasileiro. Tradicionalmente, apenas as suas raízes são utilizadas para fins comerciais, em função do seu teor conhecido de β-ecdisona. As raízes de *Pfaffia glomerata* são usadas na medicina popular brasileira, especialmente, como tónicas, afrodisíacas e antidiabéticas. Ainda, com base em dados etnobotânicos, as raízes e folhas da planta têm sido utilizadas ancestralmente como estimulantes gerais, tranquilizantes, antirreumáticas, antiarreias, anti-inflamatórias, febrífugas, cicatrizantes internos e externos, hemorroidal, na melhora da visão e da memória, para o tratamento de distúrbios gástricos, artrite, artrose, anemia, astenia e dores. Neste trabalho, as concentrações de β-ecdisona em infusões aquosas de folhas, flores, raízes e caules de indivíduos de seis meses e de três anos de idade foram determinadas por cromatografia líquida de alta eficiência. As folhas apresentaram as maiores concentrações de β-ecdisona em ambas idades (1.65 ± 0.14% para o indivíduo de três anos de idade e 1.06 ± 0.10% para o indivíduo de seis meses). As amostras de flores, de raiz e de caule do indivíduo de três anos, apresentaram: 1.14 ± 0.10%, 0.68 ± 0.05% e 0.62 ± 0.06% de β-ecdisona, respectivamente. Nas amostras do indivíduo de seis meses foram observados teores de 0.57 ± 0.02%, 0.51 ± 0.12% e 0.43 ± 0.12% para flores, raiz e caule, respectivamente. Os resultados sugerem que as partes aéreas da planta também podem ser de interesse comercial, visto que as folhas apresentaram as maiores concentrações que as demais partes da planta em ambas as idades.


1 INTRODUCTION

Technologies and management practices that add value to local production systems are of fundamental importance for sustainable territorial development (FERNANDES et al., 2016). Sustainable production systems, farming practices and forest use are global challenges addressed by the 2030 Agenda for Sustainable Development of the United Nations (ONU, 2015). A strategy that encompasses these three issues is the use of natural plant products. Natural products are used to promote health and well-being, whether in drugs, food supplements, cosmetics, teas and aromatherapy (PINTO et al., 2002; 2003). This use, which has been used for a long time, currently has science as an ally and reinforces the importance of biodiversity conservation, as we know that the medicinal and therapeutic properties of many plants and animals have not yet been discovered and that they contain potential cures for many diseases that plague the world. Natural products are the only therapeutic resource for part of the Brazilian population, and for more than 2/3 of the planet's population. According to Pinto e colaboradores (2002) the world herbal medicine market
billions per year and has been attracting more followers in developed countries every year. He points out that a serious problem in the sale and export of herbal medicines in Brazil is the lack of the status of ethical medicine that guarantees effectiveness, safety and quality, standards that are measured in scientific bases for the safety of the user. Thus, endemic medicinal plants are still little known and constitute a promising subject of academic research and development.

_Pfaffia glomerata_ (Spreng.) Pedersen, one of the _Pfaffia_ (Amaranthaceae) species identified as Brazilian ginseng, is traditionally cultivated by small-scale producers in Brazil. _P. glomerata_ production plays an important role in local economy, culture and biodiversity conservation (GODOY, 2001). Brazil is one of the major suppliers of _P. glomerata_ roots for medicinal, nutritional and cosmetic purposes (GOMES et al., 2006). In the Paraná state, _P. glomerata_ is grown in an area of environmental protection, on islands and floodplains in the Paraná River. Local farmers are organized in Association of Small-Scale Producers of Brazilian Ginseng (ASPAG) localized at Querência do Norte city, Paraná state. ASPAG promotes good farming, harvesting and processing practices with the aim of enhancing sustainability and adding value and diversity to the supply chain of _P. glomerata_ (CORRÊA-JÚNIOR et al., 2006).

_P. glomerata_ is a hydrophyte (grows partially or completely underwater) and heliophyte (prefers full sunlight) that occurs in riparian forests and floodplains (SMITH; DOWNS, 1998). The plant grows well in sandy soil and soil rich in organic matter and may develop in clay soils (MAGALHÃES, 2000). The species is distributed across tropical and subtropical South America, from Guyana to Bolivia and Argentina. In Brazil, it occurs mainly in Paraná and Mato Grosso do Sul states (TESKE; TRENTINI, 2001).

The common name Brazilian ginseng originates from the morphological similarity between _P. glomerata_ roots and those from Asian ginseng ( _Panax ginseng_, Araliaceae). Both plants have similar medicinal uses (NEVES et al., 2016; VARDANEGA, 2017) but differ in chemical composition (ROSTAGNO et
al., 2014). *P. glomerata* is a cure-all plant used in folk medicine to treat several diseases because of its analgesic, anti-inflammatory, tonic, aphrodisiac, antidiabetic, gastoprotective and antimicrobial properties (NETO et al., 2005; VARDANEGA, 2019; VARDANEGA, 2017; ROSTAGNO, 2014). These positive health effects are mainly associated with the presence of β-ecdysone (2β,3β,14α,20β,22,25-hexahydroxy-7-cholesten-6-one), C$_{27}$H$_{44}$O$_{7}$.

β-Ecdysone (Figure 1) is the highest added-value compound of *P. glomerata* (FREITAS et al., 2004; NAKAMURA et al., 2010). This ecdysteroid is used as a chemical marker of *P. glomerata* root quality (DE PARIS et al., 2000; FREITAS et al., 2004; NETO et al., 2005; MAZZEO, 2015). Takemoto and co-workers (1967) were the first to identify β-ecdysone in plants, in *Achyranthes fauriei* roots. A few years later, β-ecdysone was isolated from the roots of *P. glomerata* and, since then, the plant has been considered an important source of this bioactive compound (SHIOBARA et al., 1993).

![Figure 1 - Structural formula of β-ecdysone (C$_{27}$H$_{44}$O$_{7}$).](image)

Traditionally, only the roots are used for commercial and medicinal purposes, depending on their β-ecdysone content. However, Flores (2006) showed that the extract of both roots and aerial parts of *P. glomerata* contains β-ecdysone. Most ecdysteroids occur in low concentrations, less than 0.1% dry weight, particularly in Amaranthaceae (DINAN, 2001; REIXACH et al., 1996; SAVCHENKO et al., 1998). In *P. glomerata*, β-ecdysone contents range from...
0.15 to 0.75%, indicating a great variability in metabolite accumulation between specimens (FREITAS et al., 2004; ZIMMER et al., 2006). Serra and co-workers (2012) found that methanolic extracts of *P. glomerata* flowers had higher concentrations of β-ecdysone than those from roots. Such variations may be due to differences in analytical methods employed for the determinations, plant developmental stage and environmental conditions.

 Until mid-2010, *P. glomerata* producers used fire to burn plant shoots as a way of facilitating root harvest. ASPAG decided to replace this practice by cutting to reduce the socioenvironmental impacts of *P. glomerata* production. The current farming method opens the possibility of using the aerial parts of *P. glomerata* to increase yields. Considering this practice and previous findings that β-ecdysone can also be present in aerial parts of *P. glomerata*, this study aimed to assess the β-ecdysone contents in aqueous infusions of roots, stems, flowers and leaves of young and mature *P. glomerata* individuals analyzing by high-performance liquid chromatography (HPLC).

### 2 MATERIAL AND METHODS

#### 2.1 Sample collection and preparation

*P. glomerata* specimens were collected from cultivation farms on islands and floodplains of Paraná river in Querência do Norte (23°05′49.5″S 53°29′16.5″W), Paraná, Brazil. These lands are managed by local producers who are members of ASPAG. Plants aged 6 months were collected on August 22nd, 2019, and plants aged 3 years (age of maturity for root harvest) were collected on December 2nd, 2019. Plants were visually inspected for signs of physical and/or biological contamination prior to collection, and only healthy specimens were harvested. After collection, the material was separated into roots, stems, leaves and flowers (Figure 2).
Samples were dried to 10% moisture in a forced-air oven at 60 °C for about 15 h, crushed and stored separately in a freezer.

Infusions of roots, stems, leaves and flowers were prepared by mixing 1.0 g of sample with 100 mL of boiling water, allowing the mixture to rest for 15 min at room temperature, filtering through a 0.45 µm cellulose acetate membrane and immediately analyzed.

2.2 Quantification of β-ecdysone by high-performance liquid chromatography (HPLC)

The whole process for β-ecdysone quantification is summarized in the flowchart displayed in Figure 3. Quantitative measurements were carried out by HPLC, UV/Vis detector (UV-4070/75 - deuterium lamp) on a Jasco LC-4000 chromatograph with a C18 reverse-phase column (Fortis, 250 × 4.6 nm, 5 µm), according to the method reported by Serra and co-workers (2012), with slight modifications. The column was equilibrated with a 10:90 (v/v) mixture of HPLC-
grade methanol (phase A) and ultrapure water (phase B) before each run. Separation was achieved by gradient elution at a flow rate of 1 mL min\(^{-1}\). Elution conditions were as follows: 0–5 min, 10–70% A; 5–12 min, 70% A; and 12–15 min, 70–100% A. The injection volume was 20 µL, and the absorbance was read at 245 nm. β-ecdysone was identified by comparison of its retention time with that of an external standard (20-hydroxyecdysone, ≥93%, Sigma–Aldrich) and the contents were calculated according to a standard curve. Analyses were performed in triplicate for each plant age and organ, with a total of 24 samples (2 plant ages × 4 organs × 3 determinations).

**Figure 3** – Process steps for the determination of β-ecdysone in *Pfaffia glomerata*.

The standard curve was obtained by the analysis of six concentrations of β-ecdysone (30-250 mg L\(^{-1}\)) in triplicate. Peak areas were plotted as a function of β-ecdysone concentration and the best-fit line was determined by linear regression.

Limits of detection (LOD) and quantification (LOQ) were calculated from the standard curve using Equations (1) and (2) (ICH, 2005):

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LOD = 3 SD/S Equation (1)

LOQ = 10 SD/S Equation (2)

Where SD is the standard deviation of y-intercepts (n = 3) and S is the slope of the standard curve.

3 RESULTS AND DISCUSSION

Chromatographic analysis showed that β-ecdysone was the major component in aqueous infusions prepared from *P. glomerata* roots, stems, leaves and flowers of 3-years-old (Figure 4) and of 6-month-old (Figure 5). The compound had a retention time of 9.6 min.

**Figure 4**–Chromatograms of *Pfaffia glomerata* aqueous infusions of a 3-year-old: (A) Roots, (B) Leaves, (C) Flowers and (D) Stems.
Figure 5 – Chromatograms of *Pfaffia glomerata* aqueous infusions of a 6-month-old (A) Roots, (B) Leaves, (C) Flowers and (D) Stems

An external standard curve was obtained to determine the β-ecdysone content in *P. glomerata* infusions. The linear regression equation was $y = -479x - 5157$ ($R = 0.9995$) (Figure 6). The LOD and LOQ were found to be equal to 2 mg L$^{-1}$ and 8 mg L$^{-1}$, respectively.
**Figure 6** – (A) Chromatograms of β-ecdysone standard at different concentrations. (B) Standard curve.

![Chromatograms and Standard Curve](image)

**Source:** Authors, 2020.

β-ecdysone concentrations were found to be higher in adult individuals than in young or mature plants (Table 1). Although only *P. glomerata* roots are traditionally used for medicinal and commercial purposes, the highest β-ecdysone concentrations were detected in leaves.

**Table 1** – β-Ecdysone content in infusions of roots, stems, flowers and leaves of 6-month-old and 3-year-old *Pfaffia glomerata* individuals.

<table>
<thead>
<tr>
<th>Plant organ</th>
<th>β-Ecdysone content (%)</th>
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<tbody>
<tr>
<td></td>
<td>6-month-old plants</td>
</tr>
<tr>
<td>Leaves</td>
<td>1.06 ± 0.01</td>
</tr>
<tr>
<td>Flowers</td>
<td>0.57 ± 0.02</td>
</tr>
<tr>
<td>Stems</td>
<td>0.43 ± 0.12</td>
</tr>
<tr>
<td>Roots</td>
<td>0.51 ± 0.12</td>
</tr>
</tbody>
</table>

**Source:** Authors, 2020.

This is the first study in aqueous infusion to demonstrate the potential of stems, flowers and, particularly, leaves of *P. glomerata* as sources of β-ecdysone. The use of aerial parts can increase the sustainability and value of local production by diversifying products, reducing social and environmental impacts of unnecessary waste generation.
4 CONCLUSION

Aqueous extracts of the aerial parts of *P. glomerata* have significant concentrations of β-ecdysone, the major bioactive component of *P. glomerata* roots. Stems, leaves and flowers are promising sources of β-ecdysone and could be availed to increase diversity and sustainability to the *P. glomerata* production chain, using the aerial parts as the roots are used, instead of discarding them.

This research contributes to the objectives of the 2030 Agenda for Sustainable Development by identifying a strategy for innovating and adding value to the *P. glomerata* production chain in Paraná, Brazil. The study also highlights the importance of bringing together research institutions and producer cooperatives in order to promote territorial development.

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