

Article

## Phytochemical Analysis of *Pfaffia glomerata* Inflorescences by LC-ESI-MS/MS

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External Editor: Derek McPhee

Received: 4 August 2014; in revised form: 15 September 2014 / Accepted: 22 September 2014 / Published: 29 September 2014

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**Abstract:** *Pfaffia glomerata* contains high levels of  $\beta$ -ecdysone, which has shown a range of beneficial pharmacological effects. The present study demonstrated that inflorescences of *P. glomerata* contain other important bioactive compounds in addition to  $\beta$ -ecdysone. The identification of compounds from inflorescences using liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) was performed for the first time. The eight compounds identified were  $\beta$ -ecdysone, flavonoid glycosides such as quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside and kaempferol-3-*O*-(6-*p*-coumaroyl)-glucoside, oleanane-type triterpenoid saponins such as ginsenoside Ro and chikusetsusaponin IV, in addition to oleanonic acid and gluconic acid. This study provided information on the phytochemicals contained in *P. glomerata* inflorescences revealing the potential application of this plant part as raw material for the phytotherapeutic and cosmetic industries.

**Keywords:** *Pfaffia glomerata*; inflorescences; LC-ESI-MS/MS;  $\beta$ -ecdysone; flavonoid glycosides; triterpenoid saponins

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## 1. Introduction

*Pfaffia glomerata* (Spreng.) Pedersen (Amaranthaceae) is a perennial herb, traditionally known as Brazilian ginseng and its roots are widely used in Brazilian traditional medicine [1,2]. Several pharmaceutical manufacturers have produced phytopharmaceuticals containing *P. glomerata*, although only the roots have been processed to obtain the active ingredients [3,4]. The extracts show several biological properties including, gastroprotective effects [5], antioxidant activity [6,7], leishmanicidal potential [8], anti-inflammatory activity, and analgesic effect [9].

Several important compounds have been isolated and identified from roots of *P. glomerata*, such as glomeric acid (a triterpenoid) and pfameric acid (a nortriterpenoid), together with ecdysterone ( $\beta$ -ecdysone), rubrosterone, oleanolic acid, and  $\beta$ -glucopyranosyl oleanolate [10].  $\beta$ -Ecdysone is the most important phytoecdysteroid in *P. glomerata* [11]. The  $\beta$ -ecdysone content in *P. glomerata* has been analyzed almost exclusively in roots, which contain large amounts of this compound [5,7,12]. However, recent studies have confirmed the presence of significant amounts  $\beta$ -ecdysone in all of the major parts of *P. glomerata*, including stems and inflorescences [3,13]. Many pharmacological effects are attributed to  $\beta$ -ecdysone, such as antioxidant activity, and wound-healing and skin-regenerating properties, in addition to its use in cosmetic preparations [6,14–16].

Although the phytochemical content of the roots of *P. glomerata* has been extensively investigated, information about the chemical compounds of other parts of this plant is still sparse. In this study, we analyzed *P. glomerata* inflorescences using the LC-ESI-MS/MS method, which led to the identification of important bioactive compounds. To our knowledge, this is the first report on the identification of compounds in *P. glomerata* inflorescences by LC-ESI-MS/MS analysis. This study demonstrates the potential value of these inflorescences for cosmetic and medicinal applications.

## 2. Results and Discussion

LC-ESI-MS/MS analyses were performed in positive and negative ionization modes to obtain maximum information on the composition of the inflorescences of *P. glomerata*.  $\beta$ -ecdysone was used as a standard because it is the main compound reported in *P. glomerata*.

**Figure 1.** Chemical structures of the eight compounds proposed in *P. glomerata* inflorescences by LC-ESI-MS/MS.

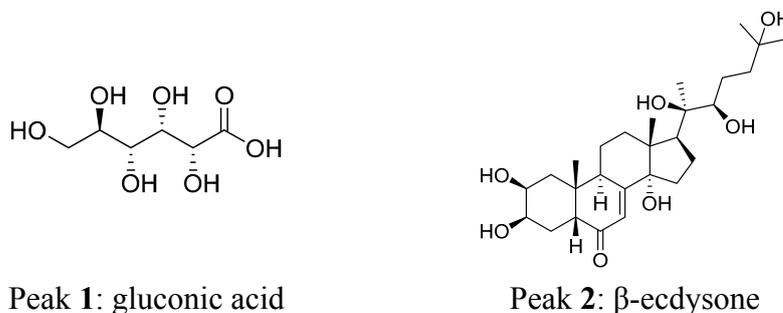
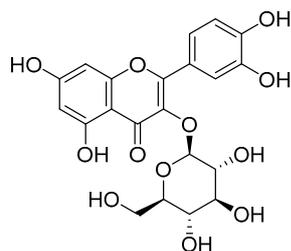
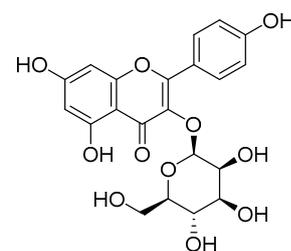
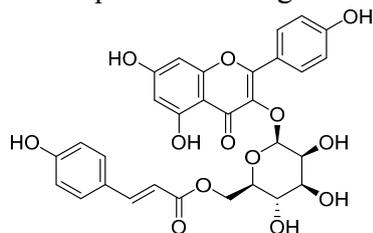
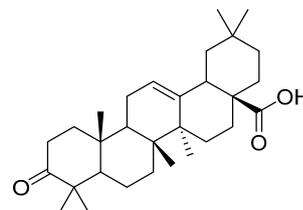
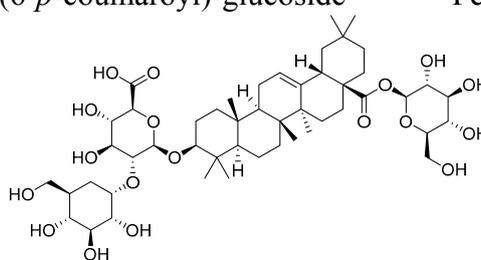


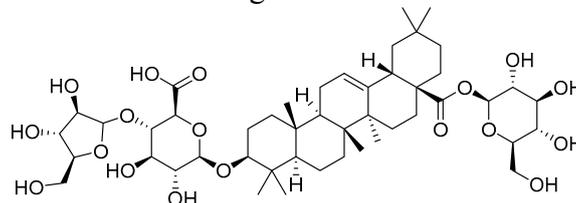
Figure 1. Cont.

Peak 3: quercetin-3-*O*-glucosidePeak 4: kaempferol-3-*O*-glucosidePeak 5: kaempferol-3-*O*-(6-*p*-coumaroyl)-glucoside

Peak 6: oleanonic acid



Peak 7: ginsenoside Ro



Peak 8: chikusetsusaponin IV

Compounds were characterized based on their mass spectra, using the precursor ion, fragment ions, and comparison of the fragmentation patterns with molecules described in the literature. Their chemical structures are shown in Figure 1, and the putative identification these compounds is discussed below and summarized in Table 1, where the compounds are numbered according to their retention times in the total ion chromatograms (TICs).

### 2.1. Identification of Compounds in *P. glomerata* Inflorescences by LC-ESI-MS/MS in Positive Ion Mode

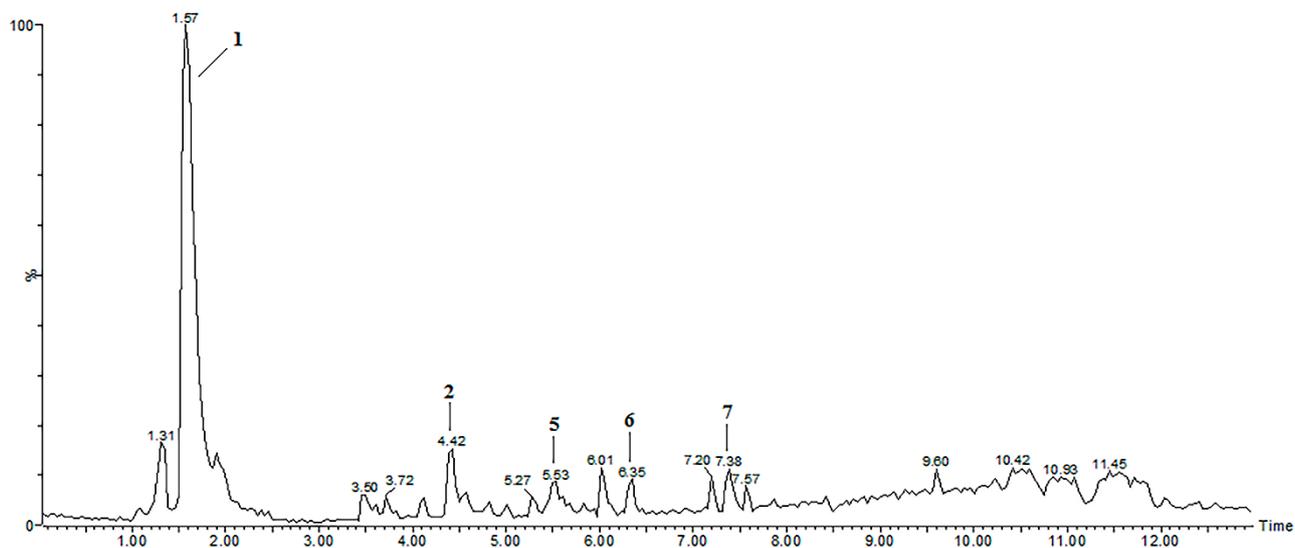
Figure 2 shows the total ion chromatogram (TIC) of the extract of *P. glomerata* inflorescences acquired in the positive ion mode. Peak 1 with a retention time ( $t_r$ ) of 1.6 min (Table 1 and Figure 2) was proposed as gluconic acid that produced an  $[M+K]^+$  ion at  $m/z$  235 by forming an adduct with a potassium ion. In addition, fragment ions at  $m/z$  118 and at  $m/z$  59 were observed in the ESI-MS/MS spectrum.

**Table 1.** Proposed compounds detected in *Pfaffia glomerata* inflorescences by LC-ESI-MS/MS in positive and negative ion modes.

Peak <sup>a</sup>	t <sub>r</sub> (min)	Positive Ion Mode		Negative Ion Mode		Tentative Assignment
		Precursor Ion (m/z)	Fragment Ions <sup>d</sup> (m/z)	Precursor Ion (m/z)	Fragment Ions <sup>d</sup> (m/z)	
1	1.6	235 [M+K] <sup>+</sup> <sup>c</sup>	<b>118</b> , 59	195 [M-H] <sup>-</sup>	177, 159, 129, 99, <b>75</b>	gluconic acid
2	4.4	481 [M+H] <sup>+</sup> <sup>b</sup>	463, 445, 427, 409, <b>371</b> , 165	525 [M+HCOO] <sup>-</sup> <sup>c</sup>	<b>479</b> , 461, 319, 159	β-ecdysone
3	4.7	ND	ND	463 [M-H] <sup>-</sup>	<b>301/300</b>	quercetin-3- <i>O</i> -glucoside
4	4.9	ND	ND	447 [M-H] <sup>-</sup>	<b>284</b>	kaempferol-3- <i>O</i> -glucoside
5	5.5	595 [M+H] <sup>+</sup>	309, 287, <b>147</b>	593 [M-H] <sup>-</sup>	447, 307, <b>285</b>	kaempferol-3- <i>O</i> -(6- <i>p</i> -coumaroyl)-glucoside
6	6.4	455 [M+H] <sup>+</sup>	438, 410, <b>147</b>	ND	ND	oleanonic acid
7	7.4	439 [aglycone+H-H <sub>2</sub> O] <sup>+</sup>	393, 249, <b>203</b> , 191	955 [M-H] <sup>-</sup>	910, <b>793</b> , 631	ginsenoside Ro
8	8.6	ND	ND	925 [M-H] <sup>-</sup>	<b>793</b> , 763	chikusetsusaponin IV

<sup>a</sup> Peak number, refer to Figures 2 and 4; <sup>b</sup> Compared with reference standard; <sup>c</sup> Adduct ions; <sup>d</sup> The base peaks in MS/MS spectra are in bold; ND: not detected.

**Figure 2.** LC-ESI-MS total ion chromatogram (TIC) of the extract of *Pfaffia glomerata* inflorescences showing peaks 1, 2, 5, 6 and 7 detected in the positive ion mode.



Kim *et al.* [17] showed that gluconic acid forms sodium adducts. In the positive mode, acids commonly form adducts with the cations in the sample or mobile phase, and in the negative ionization mode, they deprotonate easily [18]. Gluconic acid is produced from glucose, and occurs naturally in fruit, honey and wine [19,20]. Gluconic acid and its derivatives have wide applications in the food and pharmaceutical industries [19].

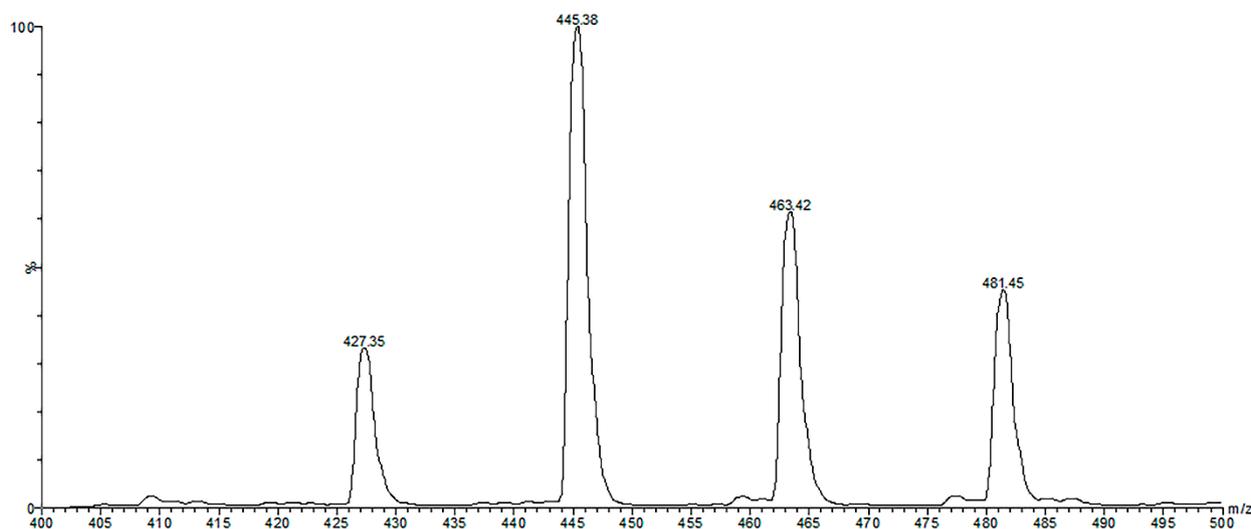
Peak 2 with  $t_r$  of 4.4 min (Table 1 and Figure 2) was identified as  $\beta$ -ecdysone with  $[M+H]^+$  ion at  $m/z$  481 and fragment ions at  $m/z$  463, 445, 427 and 409, which correspond to the successive loss of 1–4 molecules of water. This is in agreement with the ESI-MS/MS spectrum obtained for the  $\beta$ -ecdysone standard (Figure 3). These fragment ions associated with water loss were also observed in the MS/MS spectrum of  $\beta$ -ecdysone in previous studies [21–24]. Wainwright *et al.* [25] reported that for free ecdysteroids, the major ions observed in the spectra are the protonated pseudomolecular ion  $[M+H]^+$  and ions corresponding to the successive losses of water molecules,  $[M+H-nH_2O]^+$  ( $n = 1-4$ ).

This loss of water could arise from the OH group at position C-25 and C-14, which could be explained by a proton-bridging effect on the 20,22-diol function and 2,3-diol function, respectively [22,23,26]. In addition, the protonation of C25-OH followed by loss of water is favored due to the formation of a stable tertiary carbonium ion, which is also produced from the C14-OH [22,24]. Other characteristic fragment ions at  $m/z$  371 and at  $m/z$  165 were observed in the ESI-MS/MS spectrum obtained for peak 2, as also reported in previous studies [23,26,27]. The fragment ion with  $m/z$  371 was produced from cleavage between C-23 and C-24 in the side chain, followed by the loss of two molecules of water, as described by Destrez *et al.* [26].

Peak 5 with  $t_r$  of 5.5 min (Table 1 and Figure 2) was proposed as kaempferol-3-*O*-(6-*p*-coumaroyl)-glucoside (tiliroside) based on the  $[M+H]^+$  ion at  $m/z$  595 and fragment ions at  $m/z$  287 and at  $m/z$  309, which are characteristic of kaempferol and the coumaroylglucoside moiety, respectively [28–31]. Another fragment ion was observed at  $m/z$  147 [coumaroyl+H] $^+$ , which is typical of the presence of coumaroyl moieties, according to Karioti *et al.* [32]. Kaempferol-3-*O*-(6-*p*-coumaroyl)-glucoside is formed by acylation of the sugar moiety with hydroxycinnamic acid [31–33]. Previous studies showed

that acylated flavonoid glycosides have higher antioxidant and antibacterial activities than their corresponding glycosides [34].

**Figure 3.** Direct-infusion ESI-MS/MS spectrum of  $\beta$ -ecdysone standard showing  $[M+H]^+$ ,  $[M+H-H_2O]^+$ ,  $[M+H-2H_2O]^+$ , and  $[M+H-3H_2O]^+$  in positive ion mode.



Peak 6 with  $t_r$  of 6.4 min (Table 1 and Figure 2), which produced an  $[M+H]^+$  ion at  $m/z$  455 in the full MS scan, was tentatively identified as oleanonic acid. The ESI-MS/MS spectrum showed characteristic fragment ions such as  $[M+H-OH]^+$  ion at  $m/z$  438 due to the loss of a hydroxyl group, and a fragment ion at  $m/z$  410  $[M+H-CO_2H]^+$  corresponding to the loss of  $CO_2H$ , as previously described, in addition to a fragment ion at  $m/z$  147 [35].

Peak 7 with  $t_r$  of 7.4 min (Table 1 and Figure 2) was suggested as oleanolic acid aglycone based on the ESI-MS/MS spectrum, which showed a fragment ion at  $m/z$  439  $[M+H-H_2O]^+$  corresponding to the loss of one molecule of water, a fragment ion at  $m/z$  393  $[M+H-H_2O-HCO_2H]^+$  due to the loss of formic acid, in addition to fragment ions at  $m/z$  249  $[C_{16}H_{25}O_2]^+$ , at  $m/z$  203  $[C_{15}H_{23}]^+$  and at  $m/z$  191  $[C_{14}H_{23}]^+$ , which is in agreement with the literature [35–38]. Oleanolic acid and its derivatives were identified in roots of *P. glomerata* by Shiobara *et al.* [10]. The oleanane-type triterpenoid saponins have oleanolic acid as an aglycone skeleton; product ions of  $[M+H-H_2O]^+$  at  $m/z$  439 formed by retro-Diels-Alder (RDA) fragmentation in positive ion mode are useful to screen these saponins, as described by Li *et al.* [38].

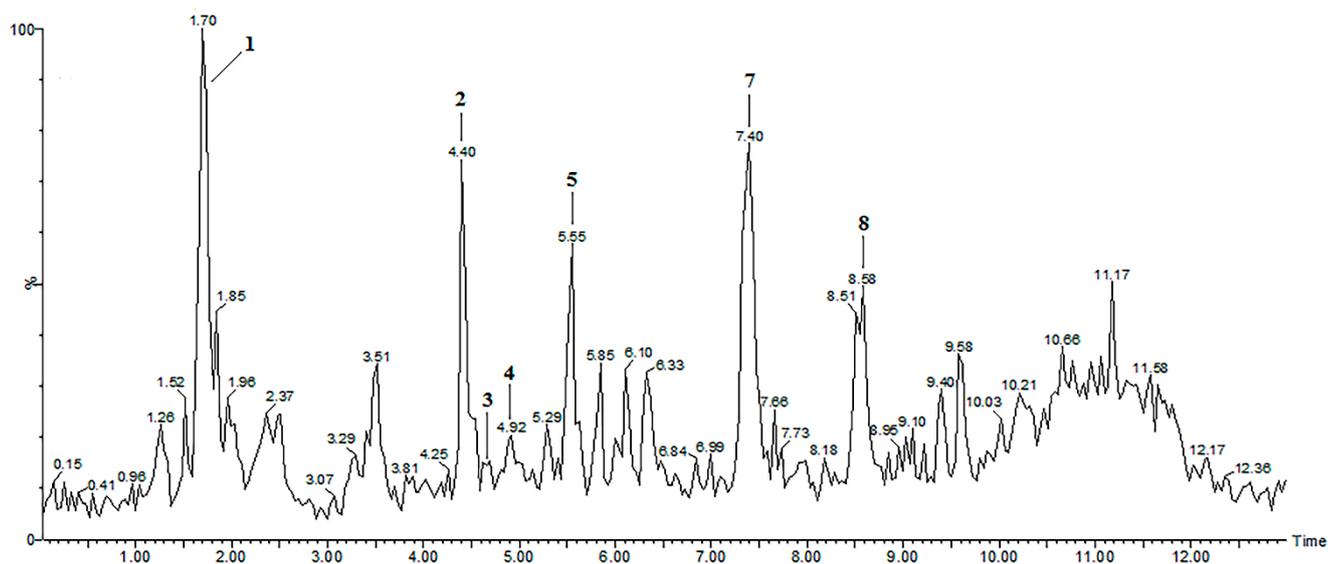
## 2.2. Identification of Compounds in *P. glomerata* Inflorescences by LC-ESI-MS/MS in Negative Ion Mode

In the LC-ESI-MS/MS analysis of the extract of *P. glomerata* inflorescences using the negative ion mode, peak 1, which was proposed as gluconic acid in the positive mode (Table 1 and Figure 4), demonstrated an  $[M-H]^-$  ion at  $m/z$  195 and fragment ions at  $m/z$  177,  $m/z$  159,  $m/z$  129,  $m/z$  99 and  $m/z$  75 in the ESI-MS/MS spectrum. These are characteristic fragment ions of this compound [39–41].

Peak 2 with  $t_r$  of 4.4 min (Table 1 and Figure 4), which as confirmed as  $\beta$ -ecdysone in the positive mode, exhibited the adduct ion  $[M+HCOO]^-$  at  $m/z$  525 in the negative mode, due to the addition of 0.1% formic acid in the mobile phase, in agreement with previous studies [23,42]. The ESI-MS/MS

spectrum showed the molecular ion  $[M-H]^-$  at  $m/z$  479, and a fragment ion at  $m/z$  461 due to the loss of one molecule of water, as described by Stevens *et al.* [23] and Destrez *et al.* [43]. Other fragment ions were observed at  $m/z$  319 and  $m/z$  159 resulting from cleavage on the side chain between C17 and C20 in  $\beta$ -ecdysone, as previously reported [23,26,43].

**Figure 4.** LC-ESI-MS total ion chromatogram (TIC) of the extract of *Pfaffia glomerata* inflorescences showing peaks 1, 2, 3, 4, 5, 7 and 8 detected in the negative ion mode.



In addition, the LC-ESI-MS/MS analysis of the extract of *P. glomerata* in the negative ion mode showed three flavonol derivatives. Peak 3 with  $t_r$  of 4.7 min (Table 1 and Figure 4) was tentatively assigned to quercetin-3-*O*-glucoside (isoquercitrin) with an  $[M-H]^-$  ion at  $m/z$  463 and a fragment ion [quercetin- $H$ ] $^-$  at  $m/z$  301 obtained after loss of a glucose unit ( $-162$  amu). This is in agreement with the ESI-MS/MS spectrum shown in previous studies on other plant species [44–49].

The ESI-MS/MS spectrum of quercetin-3-*O*-glucoside also showed a fragment ion at  $m/z$  300, which is characteristic of the deprotonated radical aglycone ion [quercetin- $H$ ] $^-$ , formed by the homolytic cleavage of the *O*-glycosidic bond, and has been proposed as indicative of quercetin glycosides [46,50]. Hvattum and Ekeberg [46] reported that flavonoid glycosides can undergo collision-induced homolytic and heterolytic cleavages of the *O*-glycosidic bond, producing deprotonated radical aglycone ( $(Y_0-H)^-$ ) and aglycone ( $Y_0^-$ ) product ions, depending on the structure of the flavonoid glycosides, and the nature and the position of the sugar substitution. In the case of quercetin-3-*O*-glucoside, the aglycone fragment appeared at  $m/z$  301 and the radical aglycone ion at  $m/z$  300.

Two peaks were tentatively assigned to kaempferol derivatives based on their ESI-MS/MS spectra producing the kaempferol aglycone at  $m/z$  285 in negative mode. Peak 4 with  $t_r$  of 4.9 min (Table 1 and Figure 4) with an  $[M-H]^-$  ion at  $m/z$  447, and a fragment ion [kaempferol-2 $H$ ] $^-$  at  $m/z$  284 resulting from the loss of a glucose moiety ( $-162$  amu), was suggested to be kaempferol-3-*O*-glucoside (astragalín). Our results are completely in agreement with those reported in previous studies [31,45,48,49,51].

Sánchez-Rabaneda *et al.* [44] demonstrated that the ESI-MS/MS spectra for flavonol *O*-glycosides such as hyperoside, isoquercitrin (quercetin-3-*O*-glucoside), quercitrin and kaempferol-3-*O*-glucoside show the deprotonated molecule  $[M-H]^-$  of the glycoside and the ion corresponding to the

deprotonated aglycone  $[A-H]^-$ , which is formed by loss of the rhamnose, glucose or galactose moiety from the glycosides. A previous study of the MS fragmentation of flavonol-3-*O*-glycosides demonstrated that the presence in the  $MS^2$  and/or  $MS^3$  of the deprotonated ion from their aglycones, at  $m/z$  284/285 and 300/301, indicates that the compounds are kaempferol-3-*O*- and quercetin-3-*O*- derivatives, respectively [52]. In our study, the compounds proposed as quercetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside were detected only in negative mode due to the acidic nature of these compounds, which made the ions more abundant and easily detected in this ionization mode, in agreement with the literature [53]. According to Gouveia and Castilho [54], the use of ESI operating in the negative mode has proven to be more selective and efficient in the characterization of phenolic compounds, even those present in trace amounts.

Peak 5 with  $t_r$  of 5.5 min (Table 1 and Figure 4) showed an  $[M-H]^-$  ion at  $m/z$  593 and a fragment ion at  $m/z$  285 [kaempferol-H] $^-$  due to the loss of a coumaroylglucoside moiety ( $-308$  amu), in addition to fragment ions at  $m/z$  447 [M-*p*-coumaroyl] $^-$  and at  $m/z$  307 [M-kaempferol] $^-$  suggesting the compound kaempferol-3-*O*-(6-*p*-coumaroyl)-glucoside, which was also tentatively identified in the positive ion mode [30–32].

As far as we are aware, no reports about flavonoid glycosides in *P. glomerata* inflorescences have previously been published. Although these three flavonoid glycosides have been proposed, their isomers could also be suggested too. According to Plazonić *et al.* [55], flavonoid glycosides have many isomers with the same molecular weight but different aglycone and sugar components at different positions on the aglycone ring. Kite and Veitch [56], reported that determining the identity of the sugars, and the manner in which they are linked, by mass spectrometry alone is challenging. For example, the loss of 162 amu indicates a hexose sugar but does not specify whether that sugar is glucose or galactose.

Peak 7 with  $t_r$  of 7.4 min (Table 1 and Figure 4), which was suggested in the positive mode to be the aglycone oleanolic acid, might be tentatively assigned to an oleanane-type triterpenoid saponin such as ginsenoside Ro (3-*O*- $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -D-glucuronopyranosyl-28-*O*- $\beta$ -D-glucopyranosyl oleanolic acid) or its isomer. The ESI-MS/MS spectrum yielded the  $[M-H]^-$  ion at  $m/z$  955, a fragment ion at  $m/z$  910 [M-H-CO<sub>2</sub>H] $^-$  due to loss of formic acid, and characteristic fragment ions at  $m/z$  793 [M-H-glucose] $^-$  and at  $m/z$  631 [M-H-2glucose] $^-$  corresponding to loss of a glucose unit ( $-162$  amu) at C-28 and to subsequent loss of another terminal glucose moiety at C-3, respectively, as reported in the literature [38,57,58]. Most fragments of triterpenoid saponins are derived from the sugar moieties in the negative mode, while the fragments originate mostly from the sapogenins (aglycones) in the positive mode [59]. The sugar moiety of oleanane-type triterpenoid saponins is important for their bioactivities [60].

The ESI-MS/MS spectrum (of the compound corresponding to peak 8 with  $t_r$  of 8.6 min (Table 1 and Figure 4), showed an  $[M-H]^-$  ion at  $m/z$  925 and fragment ions at  $m/z$  793 [M-H-132] $^-$  and at  $m/z$  763 [M-H-162] $^-$  attributed to loss of an arabinose moiety and a glucose moiety, respectively. These results suggest that the compound might be an oleanane-type triterpenoid saponin such as chikusetsusaponin IV (3-*O*- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-glucuronopyranosyl-28-*O*- $\beta$ -D-glucopyranosyl oleanolic acid) or its isomers, according to the literature [38,61]. Further investigations by NMR methods are needed to confirm the absolute configuration of the isomeric compounds, especially to define the position of the sugar moiety.

Therefore in the present study, the main compounds proposed in *P. glomerata* inflorescences were  $\beta$ -ecdysone and flavonoid glycosides, in addition to gluconic acid and triterpenoid derivatives such as saponins and oleanonic acid. If *P. glomerata* inflorescences are a source of important bioactive compounds, their utilization in phytotherapeutics and in the cosmetic industry should be encouraged.

### 3. Experimental Section

#### 3.1. Chemicals and Materials

The methanol used for sample preparation was purchased from Merck (analytical grade; Darmstadt, Germany). HPLC grade acetonitrile (Merck), ultrapure water (Milli-Q system; Millipore, Bedford, MA, USA) and formic acid (Merck) were used for mobile phase preparation in the LC-ESI-MS/MS analysis.  $\beta$ -ecdysone (Chromadex, Irvine, CA, USA) was used as a standard.

#### 3.2. Plant Material and Sample Preparation

*Pfaffia glomerata* was collected in April 2010 in Querência do Norte (23°05'02"S; 53°29'02"W), Paraná, Brazil. The plant was identified by Dr. Maria Salete Marchioretto, and a voucher specimen (PACA 107100) was deposited at the Herbarium PACA, Universidade do Vale do Rio dos Sinos, Rio Grande do Sul, Brazil. The plant was dried in a circulating-air oven at 45 °C. The inflorescences of the plant were then separated, triturated in a knife mill, and stored.

The extract obtained from the inflorescences was prepared using the Soxhlet method, with 10 g of plant material and a solution of ethanol:water (9:1 v/v) as established by Serra *et al.* [13]. The extract was filtered, and the organic solvent was removed under vacuum at 40 °C, using a rotary evaporator, and lyophilized.

The *P. glomerata* extract and  $\beta$ -ecdysone standard were dissolved in methanol at concentrations of 3000  $\mu$ g/mL and 500  $\mu$ g/mL, respectively. The solutions were filtered through a 0.45 mm membrane filter (Millipore, Bedford, MA, USA).

#### 3.3. LC-ESI-MS and LC-ESI-MS/MS Analyses

A Waters 2489 HPLC system coupled to a Micromass Quattro micro API mass spectrometer (Waters, Milford, MA, USA), triple quadrupole mass analyzer, with an electrospray ionization source (ESI) was used to perform the ESI-MS and ESI-MS/MS analyses, which were controlled using MassLynx 4.1 software (Waters, Milford, MA, USA).

The chromatographic separations were performed using a Symmetry C18 column (i.d., 3.5  $\mu$ m; 75  $\times$  4.6 mm) maintained at room temperature. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient system was as follows: 0 min 2% solvent B; 9 min 98% solvent B, kept for 1 min; 12 min 2% solvent B, remaining in this last condition for 1 min. The flow rate was 0.5 mL/min and the sample injection volume was 10  $\mu$ L.

The mass spectrometer operating conditions were: capillary voltage +2.5 kV (positive ionization mode) and -2.5 kV (negative ionization mode); cone voltage from 20 to 35 V. The source temperature and desolvation gas temperature were set at 150 and 450 °C, respectively. The cone gas flow and desolvation gas flow were 25 and 900 L/h, respectively. Spectra were recorded in positive and negative

ion modes. Full-scan spectra were acquired over the range of  $m/z$  100–1000. In the second MS, the most intense ions from the first MS spectrum were selected for the collision-induced dissociation (MS experiment). The standard solution of  $\beta$ -ecdysone was directly infused (10  $\mu\text{L}/\text{min}$ ) into the ESI interface using the positive ion mode. The MS information was interpreted and compared with spectra available in the literature.

#### 4. Conclusions

This is the first study to examine the chemical composition of *P. glomerata* inflorescences, determined by liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). The eight compounds proposed were  $\beta$ -ecdysone (peak 2), flavonoid glycosides such as quercetin-3-*O*-glucoside (peak 3), kaempferol-3-*O*-glucoside (peak 4) and kaempferol-3-*O*-(6-*p*-coumaroyl)-glucoside (peak 5), oleanane-type triterpenoid saponins such as ginsenoside Ro (peak 7) and chikusetsusaponin IV (peak 8), in addition to oleanonic acid (peak 6) and gluconic acid (peak 1). To our knowledge, this is the first report of these compounds in *P. glomerata* inflorescences, except for  $\beta$ -ecdysone. This study contributes to promote the use of this plant part in phytotherapeutics and in the cosmetic industry, due to the important biologically active compounds that were identified. The inflorescences could be used for extraction of these bioactive compounds, rather than being discarded during the processing of *P. glomerata* roots.

#### Acknowledgments

This study was financially supported by the National Council for Scientific and Technological Development (CNPq), the Federal Agency for Support and Evaluation of Graduate Education (CAPES) and the Postgraduate Program in Pharmaceutical Sciences of the State University of Maringá (PCF-UEM).

#### Author Contributions

Author D.F.F. conceived the study, performed all the experimentation, acquisition and analysis of data and drafting of the manuscript. Authors L.Z.S.B., C.P. and E.J.P. aided in the experiments and revised the manuscript. Author D.A.G.C. conceived, designed, and supervised the study, evaluated the results, and aided in drafting and revision of the manuscript. All authors read and approved the final manuscript.

#### Conflicts of Interest

The authors declare no conflict of interest.

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*Sample Availability:* Samples of  $\beta$ -ecdysone standard and extract of *P. glomerata* inflorescences are available from the authors.

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