



β -ecdysone content and antioxidant capacity in different organs of Brazilian ginseng

Lucas Dutra Zani da Silva Souza¹ Stéphane Raquel Almeida Velande da Fonseca²
Ariana Ferrari³ Daniele Fernanda Felipe^{3*}

¹Programa de Pós-graduação em Tecnologias Limpas, Centro Universitário de Maringá (UNICESUMAR), Maringá, PR, Brasil.

²Programa de Pós-graduação em Ciência, Tecnologia e Segurança Alimentar, Centro Universitário de Maringá (UNICESUMAR), Maringá, PR, Brasil.

³Programa de Pós-graduação em Tecnologias Limpas, Centro Universitário de Maringá (UNICESUMAR), Instituto Cesumar de Ciência Tecnologia e Inovação (ICETI), 87050-900, Maringá, PR, Brasil. E-mail: danielle.felipe@unicesumar.edu.br. *Corresponding author.

ABSTRACT: *Plants that contain antioxidant compounds have attracted increasing interest for their vital role in the attenuation of oxidative damage caused by free radicals and in the treatment of various diseases. The present study investigated the β -ecdysone content and the antioxidant activity of Brazilian ginseng (*Pfaffia glomerata*) extracts obtained from inflorescences, stems, and roots. The *P. glomerata* extracts were tested for antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, β -carotene bleaching test, and phosphomolybdenum method. The β -ecdysone content of *P. glomerata* extracts was measured by high-performance liquid chromatography (HPLC). The *P. glomerata* inflorescences showed the strongest DPPH radical scavenging activity and the strongest antioxidant activity in the β -carotene bleaching assay and phosphomolybdenum test. The roots showed the lowest antioxidant capacity in all of the assays. The concentration of β -ecdysone in the plant organs followed the following decreasing order: inflorescences > stems > roots. The present study showed that *P. glomerata* inflorescence extract had high antioxidant capacity that could be attributed to the presence of β -ecdysone.*

Key words: *Pfaffia glomerata*, β -ecdysone, scavenging activity methods.

Teor de β -ecdisona e capacidade antioxidante em diferentes órgãos de ginseng brasileiro

RESUMO: *Plantas que contêm compostos antioxidantes têm atraído interesse crescente por seu papel fundamental na atenuação de danos oxidativos causados pelos radicais livres e no tratamento de várias doenças. O presente estudo investigou o conteúdo de β -ecdysone e a atividade antioxidante de extratos de ginseng brasileiro (*Pfaffia glomerata*) obtidos a partir das inflorescências, caules e raízes. Os extratos de *Pfaffia glomerata* foram testados para atividade antioxidante usando o método sequestrante do radical 2,2-difenil-1-picrilhidrazil (DPPH), sistema modelo β -caroteno-linoleato e método de fosfomolibdênio. O conteúdo de β -ecdisona dos extratos de *P. glomerata* foi medido por cromatografia líquida de alta eficiência (CLAE). As inflorescências de *P. glomerata* mostraram a maior atividade sequestrante de radical DPPH e a maior atividade antioxidante no ensaio β -caroteno-linoleato e no teste de fosfomolibdênio. As raízes mostraram a menor capacidade antioxidante em todos os ensaios. A concentração de β -ecdisona nos órgãos da planta seguiu a seguinte ordem decrescente: inflorescências > caules > raízes. Os resultados indicaram uma correlação positiva entre conteúdo de β -ecdisona e atividade sequestrante de radical DPPH. O presente estudo mostrou que o extrato das inflorescências de *P. glomerata* teve alta atividade antioxidante que poderia ser atribuída à presença de β -ecdisona.*

Palavras-chave: *Pfaffia glomerata*, β -ecdisona, métodos de atividade sequestrante.

INTRODUCTION

The plants are considered a major source of natural antioxidants that can produce significant effects in the treatment of various diseases (SHOKRI et al., 2018). The natural antioxidants can protect the human body from free radicals and retard the progression of many chronic diseases (ADWAS et al., 2019). These natural antioxidants in health products are being assessed by the food, cosmetics and pharmaceutical industries (KUSUMAWATI & INDRAYANTO, 2013; FIERASCU et al., 2019).

Pfaffia glomerata (Spreng.) Pedersen (Amaranthaceae), traditionally known as Brazilian ginseng, is a Brazilian medicinal plant of great economic interest because of its popularity in Brazil and its potential for exportation (NASCIMENTO et al., 2007). It has promising applications in both phytotherapy and phytomedicine (SALDANHA et al., 2013). The extract of *Pfaffia glomerata* roots has shown several biological properties, such as gastroprotective effects (FREITAS et al., 2004), leishmanicidal potential (TULMANN NETO et al.,

2005), antiinflammatory activity, and analgesic effect (TULMANN NETO et al., 2004). Moreover, studies have reported that the extract of *Pfaffia glomerata* roots has antioxidant activity (DANIEL et al., 2005; LEAL et al., 2010; BATISTA et al., 2019).

Several important compounds have been isolated and identified from roots of *P. glomerata*, such as glomeric acid (a triterpenoid) and pfameric acid (a nortriterpenoid), noroleanane-type triterpenes and oleanane-type triterpenes, together with ecdysterone (β -ecdysone), rubrosterone, oleanolic acid, and β -glucopyranosyl oleanolate (SHIOBARA et al., 1993; HAN et al., 2018). β -ecdysone (20-hydroxyecdysone) is a relevant marker compound in the extractive solution from roots of *P. glomerata* (ZIMMER et al., 2006). Previous studies have confirmed the presence of β -ecdysone in all of the major organs of *P. glomerata*, such as roots (FREITAS et al., 2004; LEAL et al., 2010), stems, and inflorescences (SERRA et al., 2012). β -ecdysone is a secondary metabolite with biotechnological, pharmacological, medical, and agrochemical applicability (FELIPE et al., 2019). Researchers have reported the antioxidant activity of this compound (DANIEL et al., 2005; LAFONT & DINAN, 2003; BATISTA et al., 2019). NSIMBA et al., (2008) showed that all of the isolated ecdysteroids in their study, including β -ecdysone, inhibited collagenase and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.

Considering the identification of β -ecdysone in *P. glomerata* inflorescences, stems, and roots (SERRA et al., 2012) and the antioxidant activity of β -ecdysone (DANIEL et al., 2005; NSIMBA et al., 2008), all of the major organs of *P. glomerata* appear to be sources of natural antioxidants. Although considerable investigations have been performed on the roots of *P. glomerata* to assess different activities, including antioxidant activity, scientific information about the antioxidant properties of other organs of this plant is still unavailable. This study aimed to evaluate the antioxidant activity of different organs of *P. glomerata* (the inflorescences, stems, and roots) to identify the plant part that has the highest antioxidant capacity.

MATERIALS AND METHODS

Plant material

Pfaffia glomerata was collected in Querência do Norte, Paraná, Brazil. The plant was collected and identified by Prof. Dra. Maria Salete Marchioreto. A voucher specimen (PACA 107100) was deposited at the Herbarium PACA at the

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, stems, and roots of the plant were then separated, triturated in a knife mill, and stored.

Preparation of *Pfaffia glomerata* extracts

The extracts obtained from the different organs of *P. glomerata* were prepared using the Soxhlet method with ethanol: water (9:1 v/v) as established by Serra et al. (2012). For the preparation of each extract, tissue samples of 10 g were used. The extracts were vacuum filtered with filter paper, and the organic solvent was removed under vacuum at 40 °C using a rotary evaporator and lyophilized. The extracts were stored at 4 °C until use.

HPLC analysis of β -ecdysone content

To quantify β -ecdysone in different organs of *P. glomerata*, we used the HPLC method that was developed and validated by SERRA et al. (2012). *P. glomerata* extracts obtained from the roots, stems, and inflorescences and standard β -ecdysone were dissolved in methanol at a concentration of 3000 μ g/ml in triplicate. The analyses were performed using a Shimadzu LC-10 liquid chromatograph equipped with a quaternary pump (LC-10 AT), manual injection valve (Rheodyne), 20 μ l loop, degasser (DGPU-14A), thermostatted column compartment (CTO-10A), ultraviolet/visible spectrum detector (SPD-10A), and controlled by CLASS VP software. A Phenomenex ODS (C18) column (5 μ m, 250.0 \times 4.6 mm) maintained at 30 °C was used for the chromatographic analysis. The separation was performed in a gradient system using a mixture of methanol:water as the mobile phase. At time 0-5 min, the methanol:water concentration ranged from 10:90 (v/v) to 70:30 (v/v). At time 5-12 min, the methanol:water concentration remained at 70:30 (v/v). At time 12-15 min, the methanol:water concentration ranged from 70:30 (v/v) to 100:0 (v/v). The detection wavelength was 245 nm, and the flow rate was 1 ml/min, with a run time of 15 min. The sample injection volume was 20 μ l. The retention time for the β -ecdysone standard was used to identify the corresponding peak in the *P. glomerata* extracts. The data used for the statistical analysis were relative to the quantification of β -ecdysone calculated based on the value of the peak area using the following regression equation described by SERRA et al. (2012): $y = 25847x - 218747$, where y is the peak area of β -ecdysone, and x is the concentration of β -ecdysone.

Antioxidant activity of P. glomerata extracts *DPPH free radical scavenging assay*

The free radical scavenging activity of the extracts of *P. glomerata* was evaluated according to the method described by BRAND-WILLIAMS, CUVELIER & BERSET (1995). A solution of DPPH (0.87 mM) in methanol was prepared daily before use. The sample solutions of each extract were prepared from stock solutions at a concentration of 1000 $\mu\text{g}/\text{ml}$ in methanol. Dilutions were made to obtain concentrations within the linear range of each extract. The stock solution of the inflorescence extract was dissolved in methanol, yielding concentrations of 25, 50, 75, 100, 125, and 150 $\mu\text{g}/\text{ml}$. The stock solution of the stem extract yielded concentrations of 50, 100, 200, 300, 400, and 500 $\mu\text{g}/\text{ml}$. The stock solution of the root extract yielded concentrations of 500, 600, 700, 800, 900, and 1000 $\mu\text{g}/\text{ml}$.

An aliquot (3 ml) of sample solution was mixed thoroughly with 300 μl of DPPH solution and then left for 30 min at room temperature in the dark. The absorbance of the resulting solutions was measured at 517 nm against a blank (3 ml sample solution and 300 μl methanol) using a spectrophotometer (Thermo Scientific, Evolution 60). Three replicates were made for each test sample. A methanolic solution that contained 3 ml methanol and 300 μl of the DPPH solution was used as a negative control. The methanolic solution of BHT (1-25 $\mu\text{g}/\text{ml}$) was used as a positive control. The DPPH radical scavenging activity (%) was calculated using the following equation described by SCHERER & GODOY (2009):

Scavenging activity (%) = $[(\text{Abs}_0 - \text{Abs}_1) / \text{Abs}_0] \times 100$
where Abs_0 is the absorbance of the negative control, and Abs_1 is the absorbance in the presence of the test compound (extract/BHT) at different concentrations. The scavenging ability of the extracts is expressed as the IC_{50} value, which is the concentration of the sample that is required to scavenge 50% of DPPH free radicals. The IC_{50} values were calculated using linear regression plots, in which the ordinate represents the scavenging activity (%), and the abscissa represents the concentration of the tested extracts. The plant extracts were considered active with an $\text{IC}_{50} < 500$ $\mu\text{g}/\text{ml}$ (CAMPOS et al., 2003; SANTOS et al., 2010).

The ability of the extracts to scavenge DPPH radicals at a concentration of 300 $\mu\text{g}/\text{ml}$ was then compared with the synthetic antioxidant BHT at the same concentration. The plant extracts were considered to have good antioxidant activity when the inhibition percentage of DPPH was $> 50\%$, as described by FAWOLE et al. (2012).

β -carotene linoleate model system

The antioxidant activity of the extracts, based on the β -carotene-linoleate model system, was determined according to the method of KUMARAN & KARUNAKARAN (2006). A solution of β -carotene was prepared by dissolving 2 mg β -carotene in 10 ml chloroform. An aliquot (2 ml) of this solution was transferred to a 100 ml flask. After chloroform was evaporated at room temperature, 40 μl linoleic acid, 400 μl Tween 80 emulsifier, and 100 ml aerated distilled water were added to the flask with vigorous shaking. Aliquots (4.8 ml) of this emulsion were transferred to different test tubes that contained 0.2 ml of the extracts at a concentration of 1000 $\mu\text{g}/\text{ml}$. As soon as the emulsion was added to each tube, zero-time absorbance was measured at 470 nm using a spectrophotometer. The tubes were placed in a water bath at 50 $^{\circ}\text{C}$ for 2 h. The tubes were then cooled, and absorbance was recorded. A blank, devoid of β -carotene, was prepared for background subtraction. The same procedure was repeated with the synthetic antioxidant BHT at the same concentration, which was used as a positive control. Antioxidant activity (%) was determined in triplicate for each extract and positive control and calculated using the following equation:

Antioxidant activity (%) = $(\beta\text{-carotene content after 2 h of assay} / \text{initial } \beta\text{-carotene content}) \times 100$

The plant extracts were considered to show weak, moderate, and strong antioxidant activity when the inhibition percentage of oxidation was $< 50\%$, between 50% and 70%, and $> 70\%$, respectively, as described by MELO et al. (2008).

Phosphomolybdenum method

The total antioxidant capacity of the extracts was evaluated using the phosphomolybdenum method according to the procedure described by PRIETO et al. (1999). An aliquot (0.3 ml) of each sample solution or ascorbic acid (200 $\mu\text{g}/\text{ml}$) was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). A typical blank solution contained 3 ml of the reagent solution and 0.3 ml methanol, the same solvent used for the sample. All of the tubes were capped and incubated in a boiling water bath at 95 $^{\circ}\text{C}$ for 90 min. After the samples were cooled to room temperature, the absorbance of the solution of each sample was measured at 695 nm against the blank using a spectrophotometer. The experiment was performed in triplicate. The antioxidant capacity of each extract was calculated based on the relative antioxidant activity of the extract relative to the

ascorbic acid standard using the following equation:
% relative antioxidant activity = $[(Abs_1 - Abs_0) / (Abs_2 - Abs_0)] \times 100$

where Abs_1 is the absorbance of the extract, Abs_0 is the absorbance of the blank, and Abs_2 is the absorbance of ascorbic acid. The results are expressed as milligrams of ascorbic acid equivalents (mg AAE/g dry extract).

Statistical analysis

All of the experiments were performed at least in triplicate, and the results are expressed as mean \pm standard deviation (SD). The IC_{50} values were calculated using linear regression analysis. For the analysis of β -ecdysone and in the antioxidant assays, the data were compared using analysis of variance (ANOVA) followed by Tukey's test. Pearson correlation analysis was performed to determine the correlation between β -ecdysone content and the antioxidant activity of the extracts. The level of significance was set at 5%, and differences were considered statistically significant at $P < 0.05$. The statistical analysis was performed using GraphPad Prism v. 5.0 software (GraphPad, San Diego, CA, USA).

RESULTS AND DISCUSSION

HPLC analysis of β -ecdysone content

β -ecdysone is a major phytoecdysteroid that has been found in species of the genus *Pfaffia*

(IAREMA et al., 2012). It has received considerable attention because of its pharmacological and medicinal properties. Phytoecdysteroid content, including β -ecdysone, is generally low in plants (DINAN, 2001), but *P. glomerata* has a high concentration of β -ecdysone (FESTUCCI-BUSELLI et al., 2008a). The present results demonstrated that β -ecdysone is the major compound in *P. glomerata* inflorescences, stems and roots (Figure 1), as has been demonstrated also by FESTUCCI-BUSELLI et al. (2008b) and SERRA et al. (2012).

Significant differences in the concentrations of β -ecdysone were found in these different organs of the plant (Table 1). The concentration of β -ecdysone in inflorescences ($91.35 \pm 0.65 \mu\text{g/ml}$) was significantly higher than in stems ($71.51 \pm 0.93 \mu\text{g/ml}$) and in roots ($48.57 \pm 0.32 \mu\text{g/ml}$).

These results corroborate the levels of β -ecdysone in different organs of *P. glomerata* reported by SERRA et al. (2012), who also found a higher β -ecdysone content in inflorescences than in stems and roots. In another study (FESTUCCI-BUSELLI et al., 2008b), the analysis of β -ecdysone in *P. glomerata* germplasm bank accessions demonstrated that β -ecdysone was constantly detected in flowers, roots, leaves, and stems, with the highest content in flowers. Stems had the lowest β -ecdysone content.

Several studies have evaluated the β -ecdysone content in roots of *P. glomerata*, being

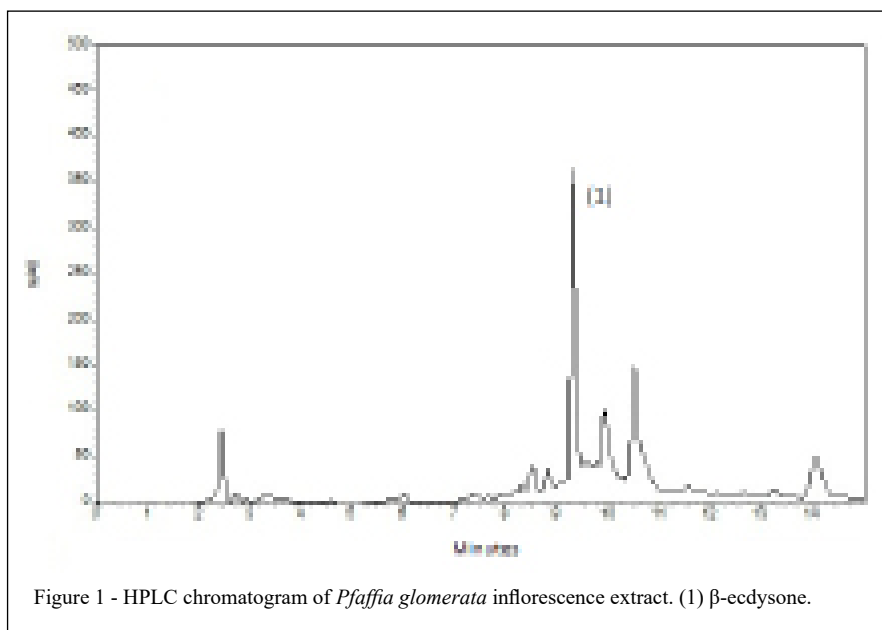


Figure 1 - HPLC chromatogram of *Pfaffia glomerata* inflorescence extract. (1) β -ecdysone.

Table 1 - β-ecdysone content in *P. glomerata* inflorescences, stems, and roots.

Extract	β-ecdysone (μg/ml)
Inflorescences	91.35 ± 0.65 ^a
Stems	71.51 ± 0.93 ^b
Roots	48.57 ± 0.32 ^c

The data are expressed as the mean ± standard deviation of triplicate measurements. Means with different letters (a-c) in the same column are significantly different ($P < 0.05$, ANOVA).

the roots widely used in Brazilian traditional medicine (FREITAS et al., 2004; ZIMMER et al., 2006; FESTUCCI-BUSELLI et al., 2008a LEAL et al., 2010). However, in these previous studies, the β-ecdysone content in roots was lower than the content in inflorescences found in the present study. Moreover, *P. glomerata* produces a large quantity of inflorescences during almost all months of the year (LEITE et al., 2005). Thus, if *P. glomerata* inflorescences are a rich source of β-ecdysone and they are often discarded during plant processing, then their utilization should be encouraged.

Furthermore, according to FELIPE et al. (2014), *P. glomerata* inflorescences contain other important bioactive compounds in addition to β-ecdysone, such as flavonoid glycosides, 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.

Antioxidant activity of *P. glomerata* extracts

Antioxidant activity is measured indirectly by determining the inhibition rate of

oxidation processes in the presence of an antioxidant (ANTOLOVICH et al., 2002). Various published methods are used to measure antioxidant capacity *in vitro*, but no single assay is capable of determining the total antioxidant ability of a studied sample. More than one method is needed to take into account the various modes of antioxidants' actions (ALAM et al., 2013). Thus, the antioxidant activity of *P. glomerata* extracts was evaluated using different assays.

DPPH free radical scavenging activity

The DPPH stable free radical method is an easy, rapid, and sensitive way to determine the antioxidant activity of a specific compound or plant extract (KOLEVA et al., 2002). This assay is based on the principle that DPPH is able to decolorize in the presence of free radical scavengers (antioxidants). The color turns from purple to yellow when the odd electron of the DPPH radical becomes paired with hydrogen from an antioxidant to form the reduced DPPH-H. The degree of discoloration indicates the scavenging potential of the compounds in terms of hydrogen donating ability (OLAJUYIGBE; AFOLAYAN, 2011).

The ability of the *P. glomerata* extracts to scavenge DPPH radicals is presented in Table 2. A lower IC₅₀ value indicates a stronger ability of the extract to act as a DPPH scavenger (TUNG et al., 2009). The extracts are considered active at an IC₅₀ < 500 μg/ml (CAMPOS et al., 2003; SANTOS et al., 2010). Thus, *P. glomerata* inflorescences (120.23 ± 2.51 μg/ml) had the strongest scavenging activity, followed by stems (297.54 ± 1.94 μg/ml). The roots (1007.29 ± 3.08 μg/ml) had the weakest scavenging activity. A significant difference in IC₅₀ values was found between the samples, including of the positive control BHT (6.86 ± 0.45 μg/ml), which exhibited higher scavenging activity than the extracts. The degree of the radical scavenging

Table 2 - 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *Pfaffia glomerata* extracts.

Extract	IC50 (μg/ml)	% inhibition of DPPH at 300 μg/ml
Inflorescences	120.23 ± 2.51c	91.9 ± 0.40a
Stems	297.54 ± 1.94b	52.8 ± 1.17b
Roots	1007.29 ± 3.08a	11.5 ± 0.85c
BHT*	6.86 ± 0.45d	92.2 ± 0.56a

*Positive control. The data are expressed as the mean ± standard deviation of triplicate measurements. Means with different letters (a-d) in the same column are significantly different ($P < 0.05$, ANOVA).

activity of the extracts was dependent on their concentration, with a considerable increase in activity as the concentration increased. At a concentration of 300 µg/ml, the inflorescences reached the maximal scavenging activity ($91.9 \pm 0.40\%$), suggesting good DPPH radical scavenging ability, once that inhibition percentage of DPPH was above 50% (FAWOLE et al. 2012) and there was no significant difference compared with the control positive BHT ($92.2 \pm 0.56\%$). At the same concentration of 300 µg/ml, the scavenging activity was significantly lower in the stems ($52.8 \pm 1.17\%$) and roots ($11.5 \pm 0.85\%$). This can be attributed to the lower β-ecdysone content in these organs of the plant, as discussed below.

The results presented in tables 1 and 2 show a positive correlation between higher β-ecdysone content in the extracts and stronger DPPH scavenging activity ($r = 0.947$, $p < 0.01$). This conclusion is also supported by published reports that demonstrated that β-ecdysone possesses strong DPPH scavenging ability, with an IC_{50} value of 3.8 ± 1.6 µg/ml (NSIMBA et al., 2008), which is higher activity than the well-known antioxidant compound BHT, which was used in our study. These researchers also reported that the antioxidant activity of β-ecdysone could be explained by the fact that its molecular structure has hydroxyl groups (hydrogen donor groups) and a methyl group (electron donor group) to its carbon skeleton. AWAH et al. (2012) reported a direct correlation between the degree of hydroxylation of the bioactive compounds and DPPH radical scavenging activity. Therefore, the strong DPPH scavenging activity of *P. glomerata* inflorescences (Table 2) may be attributed to the high β-ecdysone content (Table 1), although other antioxidant compounds may also be present in this part of the plant.

The present study compared the antioxidant properties of different organs of *P. glomerata*, namely the inflorescences, stems and roots. Previous studies had already reported the antioxidant activity of *P. glomerata* roots (DANIEL et al., 2005, LEAL et al., 2010). DANIEL et al. (2005) demonstrated the free radical scavenging activity of the butanolic fraction obtained from the roots, which was able to decrease thiobarbiturate reactive substances (TBARS) similarly to α-tocopherol, which was used as a positive control.

β-carotene linoleate model system

The β-carotene bleaching model simulates the oxidation of membrane lipid components and measures antioxidant activity toward linoleic acid (FERREIRA et al., 2006). β-carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. This is because of

the coupled oxidation of β-carotene and linoleic acid, which generates free radicals (KUMARAN; KARUNAKARAN, 2006). Linoleic acid free radical reacts with unsaturated β-carotene molecules, resulting in the oxidation of β-carotene and subsequently the loss of its characteristic orange color. However, the presence of antioxidants can hinder the extent of β-carotene bleaching by neutralizing linoleate free radical and other free radicals formed in the system (JAYAPRAKASHA et al., 2001).

Table 3 presents the antioxidant activity of *P. glomerata* extracts measured by the β-carotene bleaching assay. In the present study, at a concentration of 1000 µg/ml, the different organs of *P. glomerata* prevented the bleaching of β-carotene to different degrees. The antioxidant activity of the inflorescences ($50.4 \pm 1.45\%$) was significantly higher than the roots ($43.7 \pm 0.84\%$), but no significant difference was found compared with the stems ($47.9 \pm 0.91\%$). Although the antioxidant potential of different organs of *P. glomerata* was significantly lower than BHT ($98.1 \pm 0.55\%$), the results indicated that inflorescences and stems have moderate antioxidant activity, once that the inhibition of β-carotene bleaching was close to 50% (MELO et al., 2008). Leal et al. (2010) studied the antioxidant activity of *P. glomerata* roots using the β-carotene bleaching assay and extracts obtained under different extraction conditions. The extract obtained with low-pressure solvent extraction showed antioxidant activity close to 35%, a lower value than the one found in our study.

Phosphomolybdenum method

The total antioxidant capacity assay is a spectroscopic method for the quantitative

Table 3 - Antioxidant activity of extracts of *Pfaffia glomerata* in the β-carotene-linoleate system.

Extract	Antioxidant activity (%) at 1000 µg/ml
Inflorescences	50.4 ± 1.45^b
Stems	47.9 ± 0.91^b
Roots	43.7 ± 0.84^c
BHT*	98.1 ± 0.55^a

*Positive control. The data are expressed as the mean ± standard deviation of triplicate measurements. Means with different letters (a-c) in the same column are significantly different ($P < 0.05$, ANOVA).

determination of antioxidant capacity through the formation of the phosphomolybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH (ALAM et al., 2013). The reducing power of a compound is associated with electron donating capacity and serves as an indicator of antioxidant activity (SIDDHURAJU et al., 2002). The total antioxidant capacity of *P. glomerata* extracts is expressed as milligrams of ascorbic acid equivalents (mg AAE/g dry extract). All of the extracts exhibited some degree of antioxidant activity, with a significant difference in the values. The highest antioxidant capacity was exhibited by the inflorescences (167.8 ± 0.32 mg AAE/g dry extract), followed by the stems (137.6 ± 0.11 mg AAE/g dry extract) and roots (123.2 ± 0.40 mg AAE/g dry extract).

The antioxidant activity might be attributed to the presence of β -ecdysone in the different organs of *P. glomerata*, since this is the majority compound. However, other compounds could contribute to the antioxidant activity evaluated. For example, the compounds identified in the inflorescences by FELIPE et al. (2014), such as flavonoid glycosides, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside and kaempferol-3-*O*-(6-*p*-coumaroyl)-glucoside, have antioxidant activity, as described by ADWAS et al. (2019), who reports that flavonoids have been found to play important roles in the non-enzymatic protection against oxidative stress.

Oxidative stress causes irreversible damage in cellular macromolecules that leads to initiation of various diseases such as atherosclerosis, ischemic heart diseases, liver diseases, diabetes, initiation of carcinogenesis, and aging (ADWAS et al., 2019). The antioxidants are important species that possess the ability to protect the body from damage caused by free radicals induced oxidative stress (AZAB et al., 2017). Natural antioxidants inhibit of reactive oxygen species production and scavenging of free radicals (AZAB et al., 2017; ADWAS et al., 2019). Thus, as the *P. glomerata* inflorescence extract is a source of antioxidants, it has potential for the prevention of aging and diseases associated with oxidative stress.

CONCLUSION

The present study demonstrates that the *P. glomerata* inflorescences had a higher content of β -ecdysone and exhibited significant antioxidant capacity compared with the stems and roots. β -ecdysone may be responsible for the

antioxidant activity observed for the different organs of the plant.

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DECLARATION OF CONFLICTS OF INTERESTS

The authors declare no conflict of interest. The founding sponsor had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors were involved in the design of experiments, study, and data analysis. All authors critically revised the manuscript and approved of the final version.

REFERENCES

- ADWAS, A. et al. Oxidative stress and antioxidant mechanisms in human body. **Journal of Applied Biotechnology & Bioengineering**, v.6, n.1, p.43-47, 2019. Available from: <https://www.researchgate.net/profile/Azab_Azab/publication/331287732_Oxidative_stress_and_antioxidant_mechanisms_in_human_body/links/5c9fd648a6fdccd460458f65/Oxidative-stress-and-antioxidant-mechanisms-in-human-body.pdf>. Accessed: Feb. 03, 2020. doi: 10.15406/jabb.2019.06.00173.
- ALAM, M. N. et al. Review on in vivo and in vitro methods evaluation of antioxidant activity. **Saudi Pharmaceutical Journal**, v.21, n.2, p.143-152, 2013. Available from: <<https://www.sciencedirect.com/science/article/pii/S1319016412000357>>. Accessed: Apr. 03, 2020. doi: 10.1016/j.jsps.2012.05.002.
- ANTOLOVICH, M. et al. Methods for testing antioxidant activity. **Analyst**, v.127, n.1, p.183-198, 2002. Available from: <<https://pubs.rsc.org/en/content/articlehtml/2002/an/b009171p>>. Accessed: Feb. 06, 2020. doi: 10.1039/B009171P.
- AWAH, F. M. et al. Free radical scavenging activity, phenolic contents and cytotoxicity of selected Nigerian medicinal plants. **Food Chemistry**, v.131, n.4, p.1279-1286, 2012. Available from: <<https://www.sciencedirect.com/science/article/abs/pii/S0308814611013951>>. Accessed: Feb. 24, 2020. doi: 10.1016/j.foodchem.2011.09.118.
- AZAB, A. E. et al. Prevention of nephropathy by some natural sources of antioxidants. **Yangtze Medicine**, v.1, n.4, p.235-266, 2017. Available from: <https://www.scirp.org/pdf/YM_2017121216094513.pdf>. Accessed: Aug. 26, 2020. doi:10.4236/ym.2017.14023.
- BATISTA, D. S. et al. Reference gene selection for qRT-PCR in Brazilian-ginseng [*Pfaffia glomerata* (Spreng.) Pedersen] as affected by various abiotic factors. **Plant Cell, Tissue and Organ Culture (PCTOC)**, v.138, n.1, p.97-107, 2019. Available from:

- <<https://link.springer.com/article/10.1007/s11240-019-01606-6>>. Accessed: Apr. 12, 2020. doi: 10.1007/s11240-019-01606-6.
- BRAND-WILLIAMS, W. et al. Use of a free radical method to evaluate antioxidant activity. **LWT-Food science and Technology**, v.28, n.1, p.25-30, 1995. Available from: <<https://www.sciencedirect.com/science/article/pii/S0023643895800085>>. Accessed: Oct. 06, 2019. doi: 10.1016/S0023-6438(95)80008-5.
- CAMPOS, M. G. et al. Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. **Journal of Agricultural and Food Chemistry**, v.51, n.3, p.742-745, 2003. Available from: <<https://pubs.acs.org/doi/abs/10.1021/jf0206466>>. Accessed: Apr. 15, 2020. doi: 10.1021/jf0206466.
- DANIEL, J. F. S. et al. Free radical scavenging activity of *Pfaffia glomerata* (Spreng.) Pederson (Amaranthaceae). **Indian Journal of Pharmacology**, v.37, n.3, p.174, 2005. Available from: <<http://www.ijp-online.com/article.asp?issn=0253-7613;year=2005;volume=37;issue=3;page=174;epage=178;aulast=de>>. Accessed: Oct. 16, 2019. doi: 10.4103/0253-7613.16215.
- DINAN, Laurence. Phytoecdysteroids: biological aspects. **Phytochemistry**, v.57, n.3, p.325-339, 2001. Available from: <<https://www.sciencedirect.com/science/article/abs/pii/S0031942201000784>>. Accessed: Nov. 12, 2019. doi: 10.1016/S0031-9422(01)00078-4.
- FAWOLE, O. A. et al. Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. **BMC complementary and alternative medicine**, v.12, n.1, p.200, 2012. Available from: <<https://link.springer.com/article/10.1186/1472-6882-12-200>>. Accessed: Jan. 22, 2020. doi: 10.1186/1472-6882-12-200.
- FELIPE, D. F. et al. Phytochemical analysis of *Pfaffia glomerata* inflorescences by LC-ESI-MS/MS. **Molecules**, v.19, n.10, p.15720-15734, 2014. Available from: <<https://www.mdpi.com/1420-3049/19/10/15720>>. Accessed: Mar. 24, 2020. doi: 10.3390/molecules191015720.
- FELIPE, S. H. S. et al. Accessions of Brazilian ginseng (*Pfaffia glomerata*) with contrasting anthocyanin content behave differently in growth, antioxidative defense, and 20-hydroxyecdysone levels under UV-B radiation. **Protoplasma**, v.256, n.6, p.1557-1571, 2019. Available from: <<https://link.springer.com/article/10.1007/s00709-019-01400-3>>. Accessed: Feb. 15, 2020. doi: 10.1007/s00709-019-01400-3.
- FERREIRA, A. et al. The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. **Journal of Ethnopharmacology**, v.108, n.1, p.31-37, 2006. Available from: <<https://www.sciencedirect.com/science/article/abs/pii/S0378874106002091>>. Accessed: Mar. 02, 2020. doi: 10.1016/j.jep.2006.04.010.
- FESTUCCI-BUSELLI, R. A. et al. Biosynthesis and potential functions of the ecdysteroid 20-hydroxyecdysone—a review. **Botany**, v.86, n.9, p.978-987, 2008a. Available from: <https://www.nrcresearchpress.com/doi/abs/10.1139/B08-049#_XvoayShKjIU>. Accessed: Nov. 23, 2019. doi: 10.1139/B08-049.
- FESTUCCI-BUSELLI, R. A. et al. Level and distribution of 20-hydroxyecdysone during *Pfaffia glomerata* development. **Brazilian Journal of Plant Physiology**, v.20, n.4, p.305-311, 2008b. Available from: <<https://www.mdpi.com/1420-3049/20/4/305>>. Accessed: Nov. 23, 2019. doi: 10.1590/S1677-04202008000400006.
- FIERASCU, R. C. et al. Recovery of natural antioxidants from agro-industrial side streams through advanced extraction techniques. **Molecules**, v.24, n.23, p.4212, 2019. Available from: <https://www.scielo.br/scielo.php?pid=S1677-04202008000400006&script=sci_arttext>. Accessed: Jan. 28, 2020. doi: 10.3390/molecules24234212.
- FREITAS, C. S. et al. Involvement of nitric oxide in the gastroprotective effects of an aqueous extract of *Pfaffia glomerata* (Spreng) Pedersen, Amaranthaceae, in rats. **Life Sciences**, v.74, n.9, p.1167-1179, 2004. Available from: <<https://pubmed.ncbi.nlm.nih.gov/14687657/>>. Accessed: Jan. 28, 2020. doi: 10.1016/j.lfs.2003.08.003.
- HAN, Y. et al. Three new noroleanane-type triterpenes from the roots of *Pfaffia glomerata*. **Journal of Asian natural products research**, v.20, n.5, p.460-466, 2018. Available from: <<https://www.tandfonline.com/doi/abs/10.1080/10286020.2017.1343820>>. Accessed: Jan. 20, 2020. doi: 10.1080/10286020.2017.1343820.
- IAREMA, L. et al. Photoautotrophic propagation of Brazilian ginseng [*Pfaffia glomerata* (Spreng.) Pedersen]. **Plant Cell, Tissue and Organ Culture (PCTOC)**, v.110, n.2, p.227-238, 2012. Available from: <<https://link.springer.com/article/10.1007/s11240-012-0145-6>>. Accessed: Aug. 24, 2020. doi: 10.1007/s11240-012-0145-6.
- JAYAPRAKASHA, G. K. et al. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. **Food chemistry**, v.73, n.3, p.285-290, 2001. Available in: <<https://www.sciencedirect.com/science/article/abs/pii/S0308814600002983>>. Accessed: Apr. 14, 2020. doi:10.1016/S0308-8146(00)00298-3.
- KOLEVA, I. I. et al. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. **Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques**, v.13, n.1, p.8-17, 2002. Available in: <<https://onlinelibrary.wiley.com/doi/abs/10.1002/pca.611>>. Accessed: Jan. 24, 2020. doi: 10.1002/pca.611.
- KUMARAN, A.; KARUNAKARAN, R. J. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. **Food chemistry**, v.97, n.1, p.109-114, 2006. Available from: <<https://www.sciencedirect.com/science/article/abs/pii/S0308814605002827>>. Accessed: Nov. 12, 2019. doi: 10.1016/j.foodchem.2005.03.032.
- KUSUMAWATI, I.; INDRAYANTO, G. Natural antioxidants in cosmetics. **Studies in natural products chemistry**, v.40, p.485-505, 2013. Available from: <<https://www.sciencedirect.com/science/article/pii/B9780444596031000151>>. Accessed: Jan. 28, 2020. doi: 10.1016/B978-0-444-59603-1.00015-1.
- LAFONT, R.; DINAN, L. Practical uses for ecdysteroids in mammals including humans: and update. **Journal of insect science**, v.3, n.1, p.1-30, 2003. Available from: <<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC524647/>>. Accessed: Mar. 12, 2020. doi: 10.1093/jis/3.1.7.
- LEAL, P. F. et al. Brazilian Ginseng extraction via LPSE and SFE: Global yields, extraction kinetics, chemical composition and antioxidant activity. **The Journal of Supercritical Fluids**, v.54,

- n.1, p.38-45, 2010. Available from: <<https://www.sciencedirect.com/science/article/abs/pii/S0896844610001129>>. Accessed: Jan. 25, 2020. doi: 10.1016/j.supflu.2010.03.007.
- LEITE, G. L. D. et al. Arthropods associated to *Pfaffia glomerata* flowers in the Montes Claros-MG. **Agropecuária Técnica**, v.26, n.2, p.178-183, 2005. Available from: <https://www.researchgate.net/profile/Mayra_Pimenta/publication/237768603_ARTROPODES_ASSOCIADOS_AS_FLORES_DE_Pfaffia_glomerata_EM_MONTES_CLAROS-MG/links/582d977c08ae004f74bc942a/ARTROPODES-ASSOCIADOS-AS-FLORES-DE-Pfaffia-glomerata-EM-MONTES-CLAROS-MG.pdf>. Accessed: Mar. 16, 2020.
- MELO, E. A. et al. Antioxidant capacity of fruits. **Revista Brasileira de Ciências Farmacêuticas**, v.44, n.2, p.193-201, 2008. Available from: <https://www.scielo.br/scielo.php?pid=S1516-93322008000200005&script=sci_arttext>. Accessed: Jan. 15, 2020. doi:10.1590/S1516-93322008000200005.
- NASCIMENTO, E. X. D. et al. Produção de biomassa de *Pfaffia glomerata* (Spreng.) Pedersen e *Plantago major* L. em cultivo solteiro e consorciado. **Ciência e Agrotecnologia**, v.31, n.3, p.724-730, 2007. Available from: <https://www.scielo.br/scielo.php?script=sci_arttext&pid=S141370542007000300019&lng=pt&nrm=iso&tng=pt>. Accessed: Feb. 17, 2020. doi: 10.1590/S1413-70542007000300019.
- NSIMBA, R. Y. et al. Ecdysteroids act as inhibitors of calf skin collagenase and oxidative stress. **Journal of Biochemical and Molecular Toxicology**, v.22, n.4, p.240-250, 2008. Available from: <<https://onlinelibrary.wiley.com/doi/abs/10.1002/jbt.20234>>. Accessed: Feb. 02, 2020. doi: 10.1002/jbt.20234.
- OLAJUYIGBE, O. O.; AFOLAYAN, A. J. Phenolic content and antioxidant property of the bark extracts of *Ziziphus mucronata* Willd. subsp. mucronata Willd. **BMC Complementary and Alternative medicine**, v.11, n.1, p.130, 2011. Available from: <<https://link.springer.com/article/10.1186/1472-6882-11-130>>. Accessed: Mar. 15, 2020. doi: 10.1186/1472-6882-11-130.
- PRIETO, P. et al. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. **Analytical Biochemistry**, v.269, p.337-341, 1999. Available from: <<https://helvia.uco.es/bitstream/handle/10396/10963/Spectrophotometric%20Quantitation%20%281%29.pdf?sequence=1&isAllowed=y>>. Accessed: Nov. 12, 2020.
- SALDANHA, C. W. et al. A CO₂-enriched atmosphere improves in vitro growth of Brazilian ginseng [*Pfaffia glomerata* (Spreng.) Pedersen]. **In Vitro Cellular & Developmental Biology-Plant**, v.49, n.4, p.433-444, 2013. Available from: <<https://link.springer.com/article/10.1007%2Fs11627-013-9529-5>>. Accessed: Feb. 27, 2020. doi: 10.1007/s11627-013-9529-5.
- SANTOS, A. K. L. et al. Antioxidant activity of five Brazilian plants used as traditional medicines and food in Brazil. **Pharmacognosy magazine**, v.6, n.24, p.335, 2010. Available from: <<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2992150/>>. Accessed: Jan. 25, 2020 doi: 10.4103/0973-1296.71789.
- SCHERER, R.; GODOY, H. T. Antioxidant activity index (AAI) by the 2, 2-diphenyl-1-picrylhydrazyl method. **Food chemistry**, v.112, n.3, p.654-658, 2009. Available from: <<https://www.sciencedirect.com/science/article/abs/pii/S0308814608007218>>. Accessed: Nov. 12, 2019. doi: 10.1016/j.foodchem.2008.06.026.
- SERRA, L. Z. et al. Quantification of β -ecdysone in different parts of *Pfaffia glomerata* by HPLC. **Revista Brasileira de Farmacognosia**, v.22, n.6, p.1319-1354, 2012. Available from: <https://www.scielo.br/scielo.php?pid=S0102-695X2012000600021&script=sci_arttext>. Accessed: Oct. 18, 2019. doi: 10.1590/S0102-695X2012005000114.
- SHIOBARA, Y. et al. A nortriterpenoid, triterpenoids and ecdysteroids from *Pfaffia glomerata*. **Phytochemistry**, v.32, n.6, p.1527-1530, 1993. Available from: <<https://www.sciencedirect.com/science/article/abs/pii/003194229385172N>>. Accessed: Nov. 14, 2019. doi: 10.1016/0031-9422(93)85172-N.
- SHOKRI, Z. et al. Thyroid diseases: Pathophysiology and new hopes in treatment with medicinal plants and natural antioxidants. **International Journal of Green Pharmacy (IJGP)**, v.12, n.03, p.S473-482, 2018. Available from: <<http://www.greenpharmacy.info/index.php/ijgp/article/view/2007>>. Accessed: Feb. 14, 2020. doi: 10.22377/ijgp.v12i03.2007.
- SIDDHURAJU, P. et al. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. **Food chemistry**, v.79, n.1, p.61-67, 2002. Available from: <<https://www.sciencedirect.com/science/article/abs/pii/S0308814602001796>>. Accessed: Mar. 15, 2020. doi: 10.1016/S0308-8146(02)00179-6.
- TULMANN NETO, A et al. Analgesic and anti-inflammatory activity of a crude root extract of *Pfaffia glomerata* (Spreng) Pedersen. **Journal of Ethnopharmacology**, v.96, n.1-2, p.87-91, 2005. Available from: <<https://pubmed.ncbi.nlm.nih.gov/15588654/>>. Accessed: Jan. 25, 2020. doi: 10.1016/j.jep.2004.08.035.
- TULMANN NETO, A. G. et al. Evaluation of the trypanocidal and leishmanicidal in vitro activity of the crude hydroalcoholic extract of *Pfaffia glomerata* (Amaranthaceae) roots. **Phytomedicine**, v.11, n.7-8, p.662-665, 2004. Available from: <<https://pubmed.ncbi.nlm.nih.gov/15636182/>>. Accessed: Jan. 25, 2020. doi: 10.1016/j.phymed.2003.06.005.
- TUNG, Y. et al. Free radical-scavenging phytochemicals of hot water extracts of *Acacia confusa* leaves detected by an on-line screening method. **Food Chemistry**, v.115, n.3, p.1019-1024, 2009. Available from: <<https://www.sciencedirect.com/science/article/abs/pii/S0308814609000697>>. Accessed: Feb. 10, 2020. doi: 10.1016/j.foodchem.2009.01.026.
- ZIMMER, A. R. et al. HPLC method for the determination of ecdysterone in extractive solution from *Pfaffia glomerata*. **Journal of pharmaceutical and biomedical analysis**, v.40, n.2, p.450-453, 2006. Available from: <<https://pubmed.ncbi.nlm.nih.gov/16125356/>>. Accessed: Oct. 17, 2019. doi: 10.1016/j.jpba.2005.07.016.