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Pfaffia glomerata: Gallic acid and phenolic compounds determination

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Pfaffia glomerata: determinación de compuestos fenólicos y ácido gálico

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Abstract
Pfaffia glomerata, known as ginseng-brasileiro, is a root widely used in folk medicine, being effective against inflammation, rheumatism, and fatigue, in addition to present aphrodisiacs and antidiabetics properties. Among the classes of substances reported for the specie, the phenolic acids, in special gallic acid, has shown positive activity to learning and memory, having a considerable effect against neurotoxic and pro-oxidant substances. Therefore, the article's objective was to quantify gallic acid in the chloroform and ethyl acetate fractions from P. glomerata. Thus, the aerial parts of ginseng were collected, dried and subjected to extraction with ethanol followed by fractionation with organic solvents in different polarities, obtaining the hexanic, dichloromethane ($CHCl_3$) and ethyl acetate (AcOEt) fractions. Then, gallic acid was identified by TLC and quantified by HPLC in the ethyl acetate fraction. The total phenolic content was determined in the chloroform and ethyl acetate fractions, presenting values of 70,3 mg/g and 118,6 mg/g, respectively. The gallic acid content determined by HPLC in the AcOEt fraction was 904,15 µg/g, while in the $CHCl_3$ fraction it was not possible to quantify. So, the species can be a source of phenolic compounds for possible applications in the pharmaceutical area.
Keywords: Ginseng; HPLC; Spectrophotometry.

Resumo
A *Pffafia glomerata*, conhecida como ginseng brasileiro, é uma raiz amplamente utilizada na medicina popular, sendo eficaz no combate a inflamações, reumatismo, fadiga, além de apresentar propriedades afrodisíacas e antidiabéticas. Dentre as classes de substâncias relatadas para a espécie, os ácidos fenólicos, em especial o ácido gálico, têm demonstrado atividade positiva em relação ao aprendizado e memória, desempenhando efeito considerável frente a substâncias neurotóxicas e pró-oxidantes. Assim, o objetivo do trabalho foi quantificar o ácido gálico presente nas frações clorofórmica e acetato de etila de *P. glomerata*. Dessa forma, as partes aéreas do ginseng foram coletadas,

secas e submetidas a extração com etanol seguida de fracionamento por solventes orgânicos com diferentes polaridades, obtendo as frações hexânica, diclorometânica ($CHCl_3$) e acetato de etila (AcOEt). Na sequência, o ácido gálico foi identificado por CCD e quantificado em CLAE na fração acetato de etila. O teor de fenólicos totais foi determinado nas frações clorofórmica e acetato de etila, apresentando valores de 70,3 mg/g e 118,6 mg/g, respectivamente. O teor de ácido gálico determinado por CLAE na fração AcOEt foi de 904,15 µg/g, já na fração $CHCl_3$ não foi possível quantificar. Desta forma, a espécie pode ser uma fonte de compostos fenólicos para possíveis aplicações na área farmacêutica.
Palavras-chave: Ginseng; CLAE; Espectrofotometria.

Resumen
Pffafia glomerata, conocido como ginseng brasileño, es una raíz ampliamente utilizada en la medicina popular, siendo eficaz en la lucha contra la inflamación, el reumatismo, fatiga, además de presentar propiedades afrodisíacas y antidiabéticas. Entre las clases de sustancias notificadas para la especie, los ácidos fenólicos, especialmente el ácido lálico, han demostrado actividad positiva en relación con el aprendizaje y la memoria, teniendo un efecto considerable sobre las sustancias neurotóxicos y prooxidantes. Así, el objetivo de este trabajo fue cuantificar el ácido cónico presente en las fracciones de cloroformo y acetato de etilo de *P. glomerata*. Así, las partes aéreas del ginseng fueron recolectadas, secadas y sometidas a extracción de etanol seguido de fraccionamiento por solventes orgánicos con diferentes polaridades, obteniéndose las fracciones de hexaénica, diclorometania (CHCl3) y acetato de etilo (AcOEt). A continuación, el ácido sálico fue identificado por CCD y cuantificado en HPLC en la fracción de acetato de etilo. El contenido fenólico total se determinó en las fracciones de cloroformo y acetato de etilo, presentando valores de 70,3 mg/g y 118,6 mg/g, respectivamente. El contenido de ácido maníaco determinado por HPLC en la fracción AcOEt fue de 904,15 µg/g, mientras que en la fracción CHCl3 no fue possible cuantificar. Así, la especie puede ser una fuente de compuestos fenólicos para posibles aplicaciones en el área farmacéutica.
Palabras clave: Ginseng; HPLC; Espectrofotometría.

1. Introduction

The *Pffafia glomerata* specie, commonly known as Brazilian Ginseng, belongs to the Amaranthaceae family and has a wide distribution in Central and South America, being found in cerrado, rupestrian fields, clear fields, forest edges, riverbanks, and capoeira biomas (Siqueira, 1988). It is native to Brazil, because it is adaptable to the humid tropical climate, and is located on the banks and islands of the Paraná River, between the states of São Paulo, Mato Grosso do Sul, and Paraná. It is an herbaceous perennial plant that can reach up to 2 m in height.

Popularly, *P. glomerata* is used against inflammation, rheumatism, fatigue and stress (Nicoloso, 1999), with the roots have an aphrodisiac and antidiabetic effect (Alvim et al., 2008). However, there are few pharmacological studies that prove the efficacy reported in popular medicine. According to Vigo et al. (2003) and collaborators, the freeze-dried hydroalcoholic extract of the roots presented stimulant effects in mice, in addiction shows positive effects on learning and memory in aged rats.

Compounds present in similar species act in the Central Nervous System (CNS) activating neuroprotective mechanisms. Beyond that, they promote homeostasis, offering a greater physical and mental resistance to stress agents and enhancing cognitive functions. They are also responsible for regulating the immune system and shaping its response, besides presenting protective properties to the cardiovascular system by acting as a vasodilator (Fernandes, 2011).

The phytochemical study of *P. glomerata* shows that the species is rich in saponins, flavonoids, and phenolic acids, besides containing coumarins, lignins, and ecdysteroids (Trapp, 2018). Among the substances reported, gallic acid is a natural antioxidant, which has shown positive activity in relation to memory, besides its effect against neurotoxic and pro-oxidant substances (Brum, 2019). Thus, the aim of this study was to characterize and quantify the gallic acid present in the aerial parts of *P. glomerata* in order to assess the feasibility of applying the substance in pharmaceutical formulations in the future.

2. Methodology

2.1 Collect and drying the plant species

The *Pfaffia glomerata* aerial parts were collected on February 2021, in Toledo-PR (24°42'59.8"S 53°43'00.5"W). After collection, the plant material was dried in a fluidized bed reactor MD FBE 5.0 5L capacity, with air flow of 600 m³/h and temperature of 40 °C, enough to fluidize the material. The drying time was a total of 8 hours. Then, the leaves were manually reduced to a little size.

2.2 Fraction prepare

The ethanolic crude extract (1.58 g) was obtained by cold maceration in ethanol, and the solvent was removed at a rotary evaporator at a temperature of 30-40 ºC. Then, the crude extract was dissolved in EtOH: H_2O (1:1) and fractionated with organic solvents with different polarities: hexane (Hex), chloroform ($CHCl_3$), and ethyl acetate (AcOEt). After the removal of the solvents, the hexane (49.08 g), chloroform (53.93 g), ethyl acetate (59.4 g), and hydroethanolic (H_2O+EtOH) fractions were obtained.

2.3 Identification of gallic acid by thin-layer chromatography

The ethyl acetate fraction and the gallic acid standard were diluted in methanol (MeOH), and analyzed by TLC, with silica gel 60 F254 as the stationary phase and CHCl3:MeOH (60:40 v/v) as mobile phase. The compounds observed on the plaque were revealed in a UV light chamber at 254 nm.

2.4 Quantification of total phenolic compounds in spectrophotometer

For the quantification of total phenolic compounds in P. glomerata extract, following the method developed by Singleton, et al., (1999), the chloroform and ethyl acetate fractions were used, with Folin-Ciocalteu reagent. This method consists of a colorimetric oxidation-reduction reaction, which requires an alkaline medium for the phenolate ion to be oxidized, while the Folin reagent is reduced. The higher the concentration of total phenolic compounds in the solution, the more intense the blue coloration will be.

The operating range used for the construction of the standard curve was 1.0 to 18.0 µg/mL of gallic acid standard. In the colorimetric reaction, 1.5 mL of Na_2CO_3 and 0.5 mL of the Folin reagent were used and, after 15 minutes in a water bath (500-1D Ethik) at 40°C, the absorbance ranges of the solutions were measured in a UV-Vis spectrophotometer (SP-22 Biospectro) at a wavelength of 760 nm. The obtained absorbance values were used to calculate the concentration in µg/g of extract, using the straight-line equation obtained by linear regression.

2.5 Identification and determination of gallic acid using HPLC

The identification and quantification of gallic acid present in the ethyl acetate and chloroform fractions of P. glomerata were determined by high-performance liquid chromatography (Shimadzu), using the gallic acid standard solution at concentrations from 0.19 to 5.0 µg/mL for the construction of the standard curve. The elution mode was isocratic, using a mixture of Milli-Q water:MeOH (80:20 v/v). The chromatographic column used was Kromasil 100-5-C18 (150x4.6 mm), oven temperature 35°C, wavelength 280 nm, flow rate 0.3 mL/min, and LC Solution software.

The sample preparation was performed using Milli-Q:MeOH water (50:50 v/v), before injecting it into the chromatograph the sample was filtered through a 0.45 µm pore diameter filter. For analyte identification, the retention time of the sample was used in comparison with the retention time of the gallic acid standard. To validate the analytical method, the limit of detection (LOD) and limit of quantification (LOQ) was determined, considering the signal-to-noise (Anvisa, 2017).

3. Results and Discussion

The mobile phase was chosen according to the gallic acid standard and the AcOEt fraction behavior, which demonstrate results after an adjust in polarity. A feature of this fraction is the high polarity; therefore, it needs to be eluted in equally polar mobile phase (Souza, 2007).

The retention factor, rate between the sample eluted distance to the detriment of the mobile phase eluted distance, is the most relevant parameter in TLC, because it generates the values used to prove the presence of the determined compound (Degani, 1998).

The fraction AcOEt and the gallic acid standard, under UV 264 nm light, showed fluorescence because of the chromophore group present in this phenol (Grochanke, 2016), besides having the same retention time, under the same conditions, fact that point the gallic acid presence in the fraction. The phenolic compounds content in fraction CHCl3 was smaller (70.3 mg/g) than in fraction AcOEt (118.6 mg/g). Knowing that phenols have more affinity with higher polarity solvents, these compounds were found in higher quantities in the last fraction (Mencin et. al., 2021).

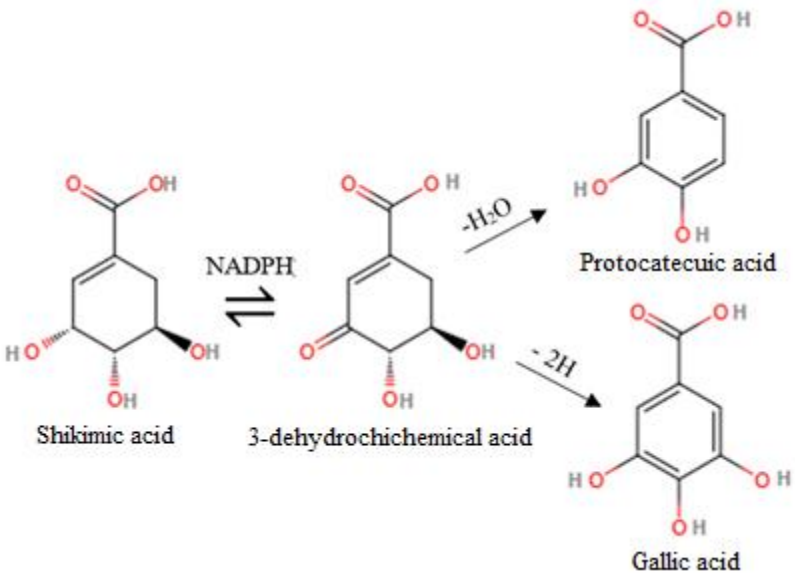
Similar results of phenolic compounds in *P. glomerata* leaves crude extract (between 180,3 and 291,0 mg EAG/L^{-1}) were verified in literature, with a 20 times reduction in root extracts (Trapp, 2018). The extraction method (Lima et. al., 2022; Baite et. al. 2021, Aquino et. al., 2020), solvent used (Sepulveda-Orellana et. al., 2021) and the interaction between extraction time and solvent has an effect on the proportion of extracted compounds (Bezerra et al., 2022).

The calibration curve of the gallic acid standard in the HPLC result in the line equation of y= 1100599x – 37962.2 with a person correlation coefficient (r) of 0.9986, which is acceptable by ANVISA (2017), which recommends $r \geq 0.99$. The retention time of gallic acid was 8 minutes, and the limits of detection and quantification were 0.06 µg/mL and 0.18 µg/mL, respectively, showing lower results compared to Lopes et al., (2009) who obtained LOD values of 0.16 and LOQ of 0.54 µg/mL for gallic acid.

The $CHCl_3$ fraction showed a peak area smaller than the quantification limit, being possible only to observe the presence of gallic acid in the fraction, not being quantifiable, since the chloroform fraction presents medium polarity (Milani et al., 2012). As for the AcOEt fraction, of high polarity, the content of gallic acid was 904.15 µg/g.

According to Franco et al. (2021), P. glomerata is rich in phenolic compounds, terpenes, alkaloids and fructan-type carbohydrates and β-ecdysone (Ribeiro et. al., 2022). The biosynthesis of most phenolic compounds, including gallic acid, occurs through the metabolic pathway of shikimic acid (Figure 1).

Figure 1 - Shikimic acid metabolic pathway.



Source: Authors.

There are few phytochemical studies of P. glomerata in literature, and other parts of the plant can be evaluated for identification and quantification of phenolic acids in future studies.

4. Final Considerations

The study of the aerial parts of P. glomerata showed that the fractionation by solvents was effective for the extraction of phenolic compounds to the ethyl acetate fraction, which presented approximately 12% of total phenolics, and 0.1% corresponds to gallic acid. Thus, P. glomerata can be a source of the phenolic compound gallic acid, for application in pharmaceutical formulations. For this, it is important to isolate and purify gallic acid from an ethyl acetate fraction of P. glomerata.

References

Alvim, N. R., da Cunha, K. C. T., Cortez, L. E. R., Bazotte, R. B., Marques, L. C., & Cortez, D. A. G. (1999). Efeitos biológicos da Pfaffia glomerata (Spreng.) Pedersen e da Pfaffia paniculata (Martius) Kuntze (Amaranthaceae). *Acta Scientiarum. Biological Sciences*, *21*, 349-352.

Aquino, T. A. C., Gualberto, N. C., Narain, N., & de Aquino Santana, L. C. L. (2020). Avaliação de compostos bioativos da casca e sementes de Sapodilla (Manilkara zapota) obtidos pela técnica de ultrassom Evaluation of bioactive compounds from Sapodilla (Manilkara zapota) peel and seeds obtained by ultrasound-assisted technique Evaluación de compuestos bioactivos de cáscara de zapote (Manilkara zapota) y semillas. *Research, Society and Development*, *9*(8), e354985158.

Baite, T. N., Mandal, B., & Purkait, M. K. (2021). Ultrasound assisted extraction of gallic acid from Ficus auriculata leaves using green solvent. *Food and Bioproducts Processing*, *128*, 1-11.

Bezerra, R. A. D., da Silva, N. M., Tuzzi, B. F., De Marchi, F. E., Feihrmann, A. C., & dos Santos, G. T. (2022). Extração de compostos fenólicos e atividade antioxidante da casca de abacate (Persea americana Mill) através de diferentes solventes. *Research, Society and Development*, *11*(13), e480111335602-e480111335602.

Brum, G. F. D. (2019). *Efeitos do tratamento com ácido gálico sobre parâmetros oxidativos, moleculares e comportamentais induzidos por cetamina em ratos*. Santa Maria: UFSM. 66 p. (Dissertação mestrado).

Degani, A. L., Case, Q. L., & Viera, P. C. (1998). Cromatografia um breve ensaio. *Química nova na escola*. (7), 21-25.

Fernandes, A. V. F. (2011). *Ginseng (Panax ginseng): Mito ou Verdade Científica?*. Portugal: Universidade Fernando Pessoa. 43 p. (Dissertação Mestrado).

Franco, R. R. (2021). *Ação neuroprotetora do ginseng brasileiro Pfaffia glomerata (Spreng.) Pedersen*. Uberlândia:UFU. 125 p. (Tese Doutorado).

Grochanke, B. S., Gehrke, I. T. S., Goettems-Fiorin, P. B., Bruxel, M. A., Basso, E. G. P., Heck, T. G., & Ludwig, M. S. (2016). Compostos fenólicos da casca de Handroanthus heptaphyllus (Mart.) Mattos e efeitos do extrato aquoso no perfil lipídico, glicêmico e na lipoperoxidação em ratos diabéticos. *Revista Brasileira de Plantas Medicinais*, *18*, 264-272.

Lima, A. B. S., dos Santos, D. O., de Almeida, V. V. S., Oliveira, A. C., & Santos, L. S. (2022). Quantification of phenolic constituents of red propolis extracts of different concentrations by HPLC. *Research, Society and Development*, *11*(8), e1111830536-e1111830536.

Lopes, G. C., Sanches, A. C. C., Toledo, C. E. M. D., Isler, A. C., & Mello, J. C. P. D. (2009). Determinação quantitativa de taninos em três espécies de Stryphnodendron por cromatografia líquida de alta eficiência. *Brazilian Journal of Pharmaceutical Sciences*, *45*, 135-143.

Mencin, M., Mikulic-Petkovsek, M., Veberič, R., & Terpinc, P. (2021). Development and Optimisation of Solid-Phase Extraction of Extractable and Bound Phenolic Acids in Spelt (Triticum spelta L.) Seeds. *Antioxidants*, *10*(7), 1085.

Milani, L. I. G., Terra, N. N., Fries, L. L. M., Cichoski, A. J., Rezer, A. P. D. S., Backes, Â. M., & Parodia, C. G. (2012). Atividade antioxidante e antimicrobiana in vitro de extratos de caqui (Diospyros kaki L.) cultivar Rama Forte. *Brazilian Journal of Food Technology*, *15*, 118-124.

Ministério da Saúde - MS - Agência Nacional de Vigilância Sanitária - ANVISA (2017). (n.d.) Resolução da Diretoria Colegiada - RDC Nº 166. http://antigo.anvisa.gov.br/documents/10181/2721567/RDC_166_2017_COMP.pdf/d5fb92b3-6c6b-4130-8670-4e3263763401#:~:text=Disp%C3%B5e%20sobre%20a%20valida%C3%A7%C3%A3o%20de .

Nicoloso, F. T., Fortunato, R. P., & Fogaça, M. A. D. F. (1999). Influência da posição da estaca no ramo sobre o enraizamento de Pfaffia glomerata (Spreng.) Pedersen em dois substratos. *Ciência Rural*, *29*, 277-283.

Ribeiro, S. T. C., da Silva, T. F. O., Castro, J. C., Cabral, M. R. P., de Abreu Filho, B. A., de Oliveira, A. J. B., & Gonçalves, R. A. C. (2022). Chemical characterization and bioactivities of fructans from Pfaffia glomerata roots. *Bioactive Carbohydrates and Dietary Fibre*, *27*, 100303.

Sepulveda-Orellana, B., Gajardo-Parra, N. F., Do, H. T., Perez-Correa, J. R., Held, C., Sadowski, G., & Canales, R. I. (2021). Measurement and PC-SAFT Modeling of the Solubility of Gallic Acid in Aqueous Mixtures of Deep Eutectic Solvents. *Journal of Chemical & Engineering Data*, *66*(2), 958-967.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in enzymology*, *299*, 152-178.

Siqueira, J. D. (1988). Considerações taxonômicas sobre as espécies brasileiras do gênero Pfaffia. *Acta Biologica Leopoldensia*, *10*(2), 269-278.

Souza, T. M., Severi, J. A., Silva, V. Y. A., Santos, E., & Pietro, R. C. L. R. (2007). Bioprospecção de atividade antioxidante e antimicrobiana da casca de Stryphnodendron adstringens (Mart.) Coville (Leguminosae-Mimosoidae). *Revista de ciências farmacêuticas básica e aplicada*, *28*(2).

Trapp, K. C. (2018). Citogenotoxicidade, fenólicos totais e crescimento de Pfaffia glomerata (Spreng.) Pedersen cultivada sob concentrações de cobre e zinco. Santa Maria:UFSM. 101 p. (Dissertação Mestrado).

Vigo, C. L. S., Narita, E., Nakamura, C. V., & Marques, L. C. (2003). Avaliação dos efeitos das raízes de Pfaffia glomerata (Spreng.) Pedersen sobre o tempo de sono e crescimento bacteriano. *Revista Brasileira de Farmacognosia*, *13*, 14-17.