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METABOLIC PROFILING OF *PLATYCODON GRANDIFLORUM* RHIZOME VIA ^{1}H NMR SPECTROSCOPY: A PRELIMINARY INVESTIGATION OF A CHINESE MEDICINAL HERB CULTIVATED IN BRAZIL

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Abstract: The rhizome of *Platycodon grandiflorum* (Balloon Flower) has been utilized as both a culinary ingredient and herbal product in Traditional Chinese Medicine. In Brazil, however, the balloon flower is primarily cultivated as an ornamental plant, resulting in scarce research on its culinary and nutraceutical properties. To enhance understanding of its metabolic profile in a tropical climate, this study analyzed the metabolome of *P. grandiflorum* rhizome extract using ^{1}H NMR spectroscopy. Thirteen primary metabolites, including sugars, amino acids, and organic acids, were identified through NMR analysis. Multivariate classification models were employed to assess the impact of cultivation method (soil or vase) and harvest season on metabolic composition. Notably, the greatest variations in metabolite concentrations — particularly amino acids and sugars — were observed between the rainy summer and the dry winter seasons. These findings suggest significant potential of *P. grandiflorum* cultivated in Brazil for both medicinal and nutraceutical applications.

Keywords: ^{1}H NMR, primary metabolism, chemometrics.

PERFIL METABÓLICO DO RIZOMA DE PLATYCODON GRANDIFLORUM VIA ESPECTROSCOPIA DE RMN DE ^{1}H: UMA INVESTIGAÇÃO PRELIMINAR DE UMA ERVA MEDICINAL CHINESA CULTIVADA NO BRASIL

Resumo: O rizoma de *Platycodon grandiflorum* (flor de balão) tem sido utilizado tanto como ingrediente culinário quanto como produto fitoterápico na Medicina Tradicional Chinesa. No Brasil, no entanto, a flor-balão é comumente cultivada como flor ornamental, sendo assim escassos os estudos acerca de seu valor nutracêutico. Com a finalidade de melhor entender o perfil metabólico desta espécie quando cultivada em clima tropical, o presente estudo analisou o metaboloma do extrato de rizomas da *P. grandiflorum* por espectroscopia de Ressonância Magnética Nuclear (RMN) de ^{1}H. Através dos espectros de RMN identificou-se 13 metabólitos primários incluindo açúcares, aminoácidos e ácidos orgânicos. Modelos de classificação multivariada permitiram avaliar a influência do tipo de cultivo (solo ou vasos) e época de colheita na produção metabólica. A variação nos teores de aminoácidos e açúcares foi a maior diferença registrada entre o verão chuvoso e o inverno seco. Os resultados baseados no perfil metabólico primário de *P. grandiflorum* cultivada no Brasil sugerem que esta espécie tem grande potencial para uso tanto alimentício quanto medicinal.

Palavras-chave: RMN de ^{1}H, metabolismo primário, quimiometria.

1. Introduction

Metabolomics is the scientific study of the metabolite composition in biological organisms. As an integral component of the broader omics disciplines, metabolomics involves analyzing the patterns of metabolites within an organism or examining the metabolic changes that occur in response to various stimuli or system modifications. In plants, the synthesis and accumulation of primary metabolites, such as amino acids, organic acids, sugars, and lipids, are closely linked to environmental factors including temperature, soil moisture, water availability, light, and seasonal variations. Sudden changes in these conditions can induce stress responses, impacting plant growth and triggering alterations in metabolic pathways. These changes can, in turn, influence species development and reproductive processes. The assessment of plant metabolites, whether through profiling or quantification, is performed using various analytical techniques. Two primary methods employed in metabolomics are nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). NMR offers advantages such as being non-destructive, requiring simple sample preparation, and providing both qualitative and quantitative analysis across a broad range of metabolites (Almeida et al., 2025; Ocampos et al., 2024; Silva Barbosa Correia et al., 2024).

P. grandiflorum (Jacq.) A. DC., balloon flower is a plant from the Campanulaceae family found naturally on the Asian continent. Its rhizomes present significant nutraceutical value and has been widely used in Asian cuisine (China, Korea and Japan) in salads, canned preparations, wines, cakes, noodles and various other food products (Lee et al., 2019; Zhang et al., 2015). The fresh rhizome contains high levels of amino acids, vitamins, zinc, calcium, iron, potassium, starch, proteins and fiber – 14.00%, 0.19% and 3.19% respectively (Huang et al., 2021; Zhang et al., 2015). This specimen also has been reported for the pharmacological properties that effectively prevent and treat lung or respiratory diseases and infections in Traditional Chinese Medicine (Zhang et al., 2015). The present study investigated the ^{1}H NMR metabolomic profile of *P. grandiflorum* rhizomes extracts cultivated in a tropical climate in Brazil throughout different seasons. The main hypothesis was observing if cultivating this specimen in a tropical climate would affect the primary metabolism production compared to similar specimens cultivated in eastern Asian countries.

2. Materials and Methods

2.1. Plant cultivation

The P. grandiflorum seedlings were purchased in 0.5 L pots at Ceagesp/SP, in 2019. After 12 months (2020), 64 plants were transplanted into the soil and distributed in three plots of 1.20 x 1.20 cm where the plants were separated by 30 x 30 cm, both in a row and in columns. The plants were grown in the area of the National Laboratory for Precision Agriculture (Lanapre) of Embrapa Instrumentation, São Carlos, located on Estrada Municipal Guilherme Scatena, at 860 m altitude 21°57'13.9" S and 47 °51 '10.9" W geographic coordinates.

2.2. Harvesting and sample preparation

The rhizomes were harvested in July 2021, in the dry/winter period (DW), and in April 2022, after the rainy/summer period (WS). Three rhizomes were randomly selected in each period. Harvesting took place regularly from 8 am. The plant material was immediately washed in distilled water and stored in a freezer at -25°C, subsequently macerated in liquid nitrogen and stored in a freezer at -25°C. Extractions were carried out in triplicate, totaling 54 samples. 50 mg of frozen macerated substrate was weighed into

eppendorfs tubes and then 750 µL of deuterated methanol and 750 µL of deuterated water (CD_3OD:D_2O) were added in a ratio of 1:1 (v/v). The eppendorfs tubes were centrifuged for 20 minutes. An aliquot of 600 µL of the obtained extracts was transferred to 5 mm tubes for NMR analysis. Reagents were purchased from Sigma-Aldrich (Darmstadt, Germany).

2.3. ^{1}H NMR metabolomics

NMR analyzes were carried out at the Brazilian Research Company - Embrapa Instrumentation in São Carlos, SP. Spectra were acquired at 25 °C on a Bruker NMR (Karlsruhe, Germany) 14.1 Tesla (600 MHz for hydrogen frequency), AVANCE III, equipped with a 5 mm PABBO direct detection probe (Broad Band Observe) with ATMA ® (Automatic Tunning Matching Adjustment), z-field gradient, BCU-I variable temperature unit, field gradient generating unit and “Sample-Xpress™” automatic sample changer. ^{1}H NMR was acquired with a NOESY pulse sequence (named noesygppr1d in TopSpin Bruker) with field gradient and suppression of the water signal by irradiation at a frequency of 2820.39 Hz (O1). Conditions were 128 averages (ns), 4 simulated sweeps (ds), 65,536 data points during acquisition (td), 20.0276 ppm spectral window (sw), fixed receiver gain (80.6 rg), pulse calibrated at 90º (13.821 µs), 2.76 s (aq) acquisition time between each acquisition, 5*T1 (19 s) waiting time between each average and 5 ms (d8) mixing time. ^{1}H NMR spectra were referenced using the TMSP-d4 signal at 0.0 ppm. Spectra were processed in Chenomx NMR software (Chenomx Inc., Edmonton, Canada) with additional metabolite quantification. The overlapping signals of each compound were deconvolved based on the Chenomx NMR software database library. Metabolites were quantified relative to the standard concentration of TSPd4. The chemical shifts metabolite assignments were confirmed according to the Human Metabolome Database (HMDB) references. Clustering patterns in the samples harvested in different seasons were investigated by partial least squares discriminant analysis (PLS-DA) through Metaboanalyst 5.0 (https://www.metaboanalyst.ca). Data was normalized by auto scaling. Validation test was conducted using Leave-One-Out-Cross-Validation along with 2000 permutations (Spricigo et al., 2023).

3. Results and Discussion

The metabolic profile was initially assessed using the spectrum of an internal quality control (QC) sample, which contains the extract of all samples. From the QC sample it was possible to assign 13 metabolites (Figure 1). The monosaccharides arabinose, galactose, ribose, xylitol and xylose exhibited characteristic doublet signals at ~3 ppm, while the disaccharides cellobiose, lactulose, maltose, sucrose and trehalose showed multiplets or triplets in the same region of spectral signals. However, sucrose exhibits a clear signal of doublets at ~5 ppm. The amino acids, arginine, glutamine and threonine were identified. After identifying the metabolites in the QC, they were assigned and quantified in the sample’s spectra.

The metabolic compounds found in this study showed similarity with other primary metabolites found in rhizomes (*i.e*. *Panax ginseng*, (Araleaceae family), *Codonopsis pilosula* (Campanulaceae family), *Beta vulgaris L*. (Amaranthaceae family), *Curcuma aromatica* and *C. longa* (family Zingiberaceae)). Compounds such as non-reducing sugars (sucrose, fructose and lactulose), reducing sugars (fructose and glucose), organic acids (malate, citrate) and amino acids (arginine, alanine, threonine, glutamine, tyrosine, leucine, proline) could also be detected. Their concentrations can vary depending on the cultivation method and environmental conditions (Liang et al., 2021; Nguyen et al., 2016; Tarachiwin et al., 2008). We proceed with PLS-DA for clustering analysis based on the concentration

of these metabolites grouping the samples by winter-21 and summer-22. PLS-DA presented group separation in the samples, an $R^2 = 0.59$, and $Q^2 = 0.4$ (Figure 2). Sugars, such as sucrose, xylitol, ribose, maltose and galactose, and Threonine were the metabolites with highest VIP scores, in other words, responsible for characterizing cluster separation.

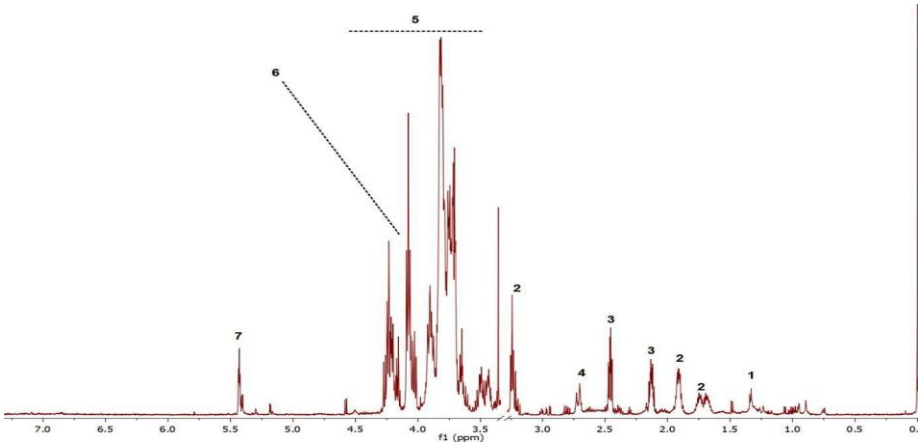

Figure 1. Spectrum for internal quality control sample. The assignment of the signals are: (1) Threonine, (2) Arginine, (3) Glutamine, (4) Aspartic Acid, (5) sugar region (galactose, maltose, threalose, xylose, arabinose, ribose, sucrose, lactulose), (6) Glyceric acid, (7) Maltose.

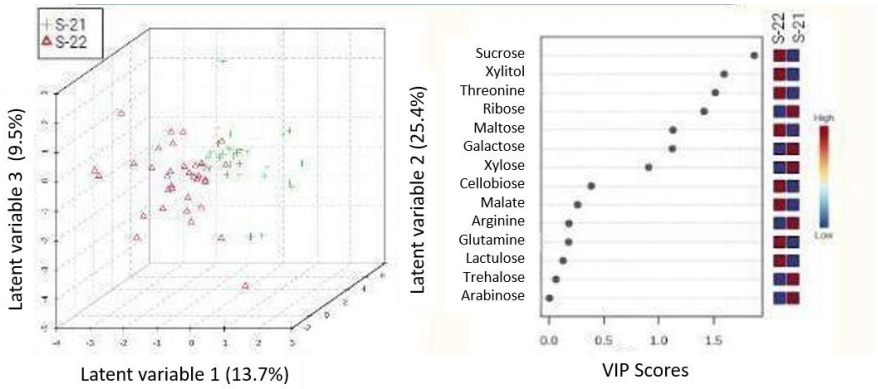

Figure 2. PLS-DA results and VIP scores. Model validation showed p <0.05 Samples harvested in winter-21 vs samples harvested in summer-22.

4. Conclusions

The primary metabolic profile of *P. grandiflorum* rhizomes is influenced by seasonal environmental conditions. To the best of our knowledge, this study represents the first characterization of the ^{1}H NMR metabolic profile of *P. grandiflorum* rhizomes cultivated in Brazil. The use of PLS-DA as a classification tool was effective in establishing grouping criteria for the samples based on primary metabolite production. Given that these metabolites are also present in other herbal products used in Traditional Chinese Medicine, we hope that our findings will contribute to future research on the cultivation of *P. grandiflorum* in tropical climates, with potential applications for both medicinal and food-related uses.

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