C-glycosyl flavones in *Passiflora incarnata*

**C-glicosil flavona em Passiflora incarnata**

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ABSTRACT
Brazil has a diversity of plant species and many of them are medicinal plants used in the production of herbal medicines. *Passiflora incarnata* is popularly known as passion fruit, its fruits and aerial parts have anxiolytic and sedative properties. Recent studies have shown that *P. incarnata* has several bioactive compounds such as indole alkaloids, cyanogenic glycosides, maltol and flavonoids. Among the substances identified in the species, vitexin has action on the Central Nervous System, interfering with the functioning of gamma-aminobutyric acid. Thus, the objective of the study was to identify and quantify the vitexin present in the ethyl acetate fraction of the aerial parts of the passion fruit. For this, the aerial parts of *P. incarnata* were submitted to extraction with ethanol, followed by fractionation in organic solvents, obtaining the hexane, dichloromethane and ethyl acetate fractions. The presence of vitexin in the ethyl acetate fraction was verified by Thin Layer Chromatography (TLC) and the total flavonoid content by colorimetric reaction in a spectrophotometer. The quantification of vitexin was performed by High Performance Liquid Chromatography (HPLC). The total flavonoid content was 5.34 mg/g and 960 µg/g of vitexin in the ethyl acetate fraction. Studies like this contribute to the development of new pharmaceutical formulations from the substance vitexin.

Keywords: *passiflora incarnata*, flavonoids, vitexin, spectrophotometer.
Camada Delgada (CCD) e o teor de flavonoides totais por reação colorimétrica em espectrofotômetro. Já a quantificação da vitexina foi realizada por CROMATOGRAFIA LÍQUIDA DE ALTA EFICIÊNCIA (CLAE). O teor de flavonoides totais foi de 5,34 mg/g e 960 μg/g de vitexina na fração acetato de etila. Estudos como esse contribuem com o desenvolvimento de novas formulações farmacêuticas a partir da substância vitexina.

**Palavras chave:** flavonóides totais, vitexina, CLAE.

1 INTRODUCTION

The passion fruit, species with the scientific name *Passiflora incarnata* L., is used medicinally in the preparation of teas, tinctures and herbal supplements that help relieve stress, combat anxiety and insomnia (ANTONIO, 2014; SANTOS; GALINDO; QUEIROZ, 2020).

About 80% of the medicines in the middle of the 20th century were derived from plants (GILANI; RAHMAN, 2005). According to the Brazilian National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária - ANVISA), there are 24 registered drugs, 17 described as *Passiflora incarnata*, 07 as *Passiflora alata* (BRAZIL, 2021).

The presence of *P. incarnata* in a range of medicines is due to the presence of different bioactive compounds, such as flavonoids, indolic alkaloids, cyanogenic glycosides and maltol (PEREIRA, 2014). Among them, there are flavonoids, especially vitexin (5,7,4’-trihidrox-8-C-glycopyranosyl flavone). The C-glycosyl flavone interferes with the functioning of gamma-aminobutyric acid, GABA, which is a neurotransmitter that inhibits the central nervous system and helps regulate the sleep cycle, improves physical and mental disposition, and reduces anxiety and stress (SILVA, 2015).

According to (ANVISA 2021), the aerial parts (leaves and stems) are used to obtain extracts, with the fractionation of these, the compound of interest is separated, thus enabling the development of drugs.

Thus, the objective of the work was to determine and quantify the vitexin in the aerial parts of *P. incarnata* for future applications in pharmaceutical forms.
2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

The aerial parts of *Passiflora incarnata* were collected in the municipality of Toledo - Paraná (23°43’12”S 53°44’36”W), on 09/02/2022 at night. The plant material was previously selected in order to remove the petioles, bruised leaves and any foreign body still present. The leaves were dried in an oven with forced air circulation at 40°C for 6 hours, being turned to a homogeneous drying. The dried material has been manually reduced to smaller sizes.

2.2 PREPARATION OF CRUDE EXTRACT AND FRACTIONATION

Approximately, 16 g of the plant material was submitted to extraction with 650 mL of cold ethanol, by exhaustive macerated. The solvent is removed in a rotational evaporator with a temperature of 35–40°C, obtaining the ethanolic crude extract (6.04 g).

Ethanolic crude extract was dissolved in methanol: water (1:1) and submitted to a partition with 3 times of 240 mL of each of the organic solvents: hexane, chloroform and ethyl acetate. After the removal of the solvents, the phenic, chloroform, ethyl acetate and hydroethanolic fractions were obtained.

2.3 IDENTIFICATION OF VITEXIN BY THIN LAYER CHROMATOGRAPHY (TLC)

The thin layer chromatography (TLC) technique used to confirm the presence of vitexin in the ethyl acetate fraction was performed qualitatively with the pattern. For this, 0.5 mg/mL vitexin solution in methanol and ethyl acetate fraction were applied in aluminum chromate sheets containing silica gel 60 F254. The mobile phase used AcOEt:MeOH 10% to saturate the chromatographic tank with vapors of the mobile phase, filter paper was used. After application of the samples and chromatographic race, the TLC is observed in a 254 nm UV light chamber and revealed with anisaldehyde-sulfuric and heating plate at 100 °C.

2.4 DETERMINATION OF TOTAL FLAVONOIDS CONTENT WITH SPECTROPHOTOMETER

The spectrophotometric determination of total flavonoid content in the aerial parts of *P. incarnata* is followed by pharmacopeia procedures described by Chabariberi et al. (2010), with adaptations. The determination technique is based on the absorbance
measurement, on a wavelength of 427 nm, of the complex formed between flavonoid and reagent aluminum (AlCl3), forming yellowish compounds (FU et al., 2010). The analyses were performed on a spectrophotometer (SP-22 Biospectro) and the total flavonoid content calculated from the equation of the calibration curve obtained by linear regression, constructed using vitexin pattern at different concentrations (0.3 µg/mL - 0.7 µg/mL). Part of the ethyl acetate fraction prepared in methanol and the colorimetric reaction similar to the preparation of the standard curve. The result of the total flavonoid content expressed as vitexin equivalent (µg/g). All trials were carried out in triplicate and the results presented with mean and standard deviation values.

2.5 IDENTIFICATION AND QUANTIFICATION OF VITEXIN IN HPLC - HIGH EFFICIENCY LIQUID CHROMATOGRAPHY

To determine the vitexin content in the ethyl acetate fraction, we used high-efficiency liquid chromatograph (Shimadzu) with an automatic injector, UV-VIS detector, LC-solution data reading software and Kromasil 100-5-C18 column (4.6 x 150 mm), and oven temperature maintained at 28 °C. The isocratic elution system, with the mobile phase composed of acetonitrile:water milli-Q (18:82). The injection volume is 20 µL, with the flow of the mobile phase of 0.3 mL/min, pressure 85 bar and wavelength 345 nm. The identification and quantification of the compound in the ethyl acetate fraction was performed by comparing the retention time and equation of the straight of the calibration. The standard curve was constructed from the increasing concentrations of vitexin pattern (0.042 µL/mL to 2 µL/mL) and the limits of detection (LD) and quantification (LQ) defined considering the signal-to-noise.

3 RESULTS AND DISCUSSION

The evaluation in CCD indicated the presence of vitexin in the ethyl acetate fraction, since when subjected to UV light (365 nm) it was possible to observe fluorescence of yellow coloration and retention factor (rf) similar to the standard. According to Buarque et al. (2015), vitexin is a phenolic compound soluble in water and polar solvents. For characterization of this compound, the aerial parts were subjected to extraction with ethanol, because according to Melinda et al., (2014), alcoholic solutions lead to cell wall lysis, exposing all the plant's secondary metabolites to the extracting liquid. In addition, fractionation enables the migration of substances according to the polarity of the solvents used, favoring a pre-purification of the crude extract. For the
quantification of total flavonoids content, the equation of the straight line \( y = 0.633x + 0.0046 \) with correlation coefficient \( R=0.9985 \) was used, which is adequate according to ANVISA (2017), and was obtained with the construction of the standard curve (Figure 1).

![Figure 1: Standard curve of vitexin in spectrophotometer](source)

Source: Own authorship

The result obtained in triplicate with different concentrations, serves for the verification and comparison of this experiment. The total flavonoid content found was 5.34 mg/g in the ethyl acetate fraction, using vitexin as a standard. Data described in Pharmacopée Française (1992) point to an average of 9.88 mg/g. The standard used for analysis was rutin. The use of rutin is justified because it is a D-glycosylated flavonoid, whose chemical structure is close to the glycosylated flavonoids present in the species studied. It is noted that the concentration of total flavonoids expressed as vitexin by colorimetric analysis of the AcOEt fraction was slightly lower than the sample value of the same AcOEt fraction with rutin, which may have influenced the difference in concentrations. Thus, it is evaluated that the standard has significant interference in the levels found (Pharmacopée Française 1992). With the construction of the standard curve of vitexin in CLAE and retention time of 13.3 minutes, the equation of the straight line \( y=131782x+2553 \) showed a correlation coefficient \( R=1 \), this result is adequate following the regulations of ANVISA (2017). The LD (limit of detection) found was 0.031 µg/mL and the QL (limit of quantification) was 0.042. In the ethyl acetate fraction of *P. incarnata* the vitexin content was 960 µg/g, these values vary according to the harvest time and
growing regions, being verified concentrations of 16.3 to 2301 µg/g in methanolic extract of *P. incarnata* (GRIFFITHS et al., 2013).

4 FINAL CONSIDERATIONS

The study of *P. incarnata* provided the identification and quantification of vitexin in the ethyl acetate fraction from the crude ethanol extract, demonstrating the importance of partitioning by organic solvents in the migration of flavonoids to polar fractions. In this way, the aerial parts of passion fruit can be used to obtain compounds with applications in new pharmaceutical formulations.
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