ATR-FTIR spectroscopy combined with chemometric tools for rapid distinction of *Passiflora* L. species

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ABSTRACT

Abstract: *Passiflora* spp., commonly known as passion fruit, hold significant economic value in pharmaceutical, cosmetic, and food industries, and are traditionally employed to alleviate insomnia and anxiety. Several analytical techniques are utilized for quality control and differentiation of medicinal plants with therapeutic purposes. In this study, we investigated the potential of Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy coupled with chemometric analysis as a rapid and non-invasive method for discriminating six *Passiflora* species. The ATR-FTIR fingerprint revealed a large number of vibrational absorption bands in the region at 2000 to 600 cm^{-1} typical of phenolic compounds, flavonoids, terpenoids, polysaccharides, polyesters, pectins, lignins, and lipids in *Passiflora* spp. Principal components analysis of the wavenumbers of ATR data was effective in the differentiation of *Passiflora* (*Passiflora alata* Curtis, *Passiflora cincinnata* Mast., *Passiflora setacea* DC., *Passiflora alata* Curtis var. BRS Mel do Cerrado, *Passiflora cincinnata* Mast. var. BRS Sertão Forte, and *Passiflora setacea* DC. var. BRS Pérola do Cerrado). The loadings plot revealed that the discrimination by *Passiflora* spp. fingerprint were mainly to the presence of flavonoids. These results suggest ATR-IR as a potentially reliable alternative for characterization, chemophenetic studies, and quality control purposes of medicinal plants.

Keywords*:* ATR-FTIR, *Passiflora*, fingerprint, flavonoids, chemometric, quality control.

INTRODUCTION

The genus *Passiflora* L. belongs belongs to the Passifloraceae family (Faleiro et al. 2017). This family includes around 520 species, broadly distributed across the Neotropic region, with Brazil being a notable biodiversity center for this family, harboring four genera and 138 species. Among the genera, *Passiflora* L. is the most representative of this family, with 129 native species in Brazil (Cervi et al. 2010). Apart from commercially cultivated species, wild *Passiflora* species have gained highlight for their great importance in pharmaceutical, industrial, and medicinal areas, and breeding programs (Patel et al. 2011; Pereira et al. 2019). Traditionally, some *Passiflora* species have great use in anxiety treatment due to their anxiolytic effect, acting as a nonspecific depressant of the central nervous system (Fonseca et al. 2020). Notably, species such as *Passiflora incarnata* L., *Passiflora alata* Curtis, and *Passiflora edulis* Sims are described in the Brazilian Pharmacopoeia for their medicinal proprieties (Anvisa 2019; 2021).

At present, various biological activities are reported in *Passiflora*, including antioxidant and photoprotective activities in *P. coccinea* Aubl. (Silva et al. 2020), gastroprotective activity in *P. alata* (Wasicky et al. 2015), promising immunomodulatory substances (inhibiting nitric oxide production) and antimycobacterial action in *P. caerulea* L. (Araujo et al. 2017), antinociceptive and anti-inflammatory effects in *P. cincinnata* Mast. (De Lavor et al. 2018), as well as potential anti-inflammatory and antidiabetic

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effects reported for *P. setacea* DC. (Duarte et al. 2020). These diverse biological activities are attributed to the presence of different classes of secondary metabolites in *Passiflora* species, highlighted alkaloids, flavonoids, cyanogenic glycosides, carotenoids (Patel et al. 2011; Ayres et al. 2015; Guimaraes et al. 2020), steroids, saponins (Lopes et al. 2017), and terpenoids (Araujo et al. 2017). Such bioactive compounds have been the subject of extensive investigation, contributing to the understanding of pharmacological actions and potential therapeutic applications of *Passiflora* species.

The scientifically investigated medicinal use of only a few species of *Passiflora* extensively, coupled with the taxonomic complexity of this genus, propensity for morphological variation and diversity of metabolites, represent challenges for the rapid discrimination and classification of *Passiflora* spp. (Plotze et al. 2005; Pérez and d'Eeckenbrugge. 2017). Thus, the challenges involving these aspects can be addressed by employing analytical techniques integrated with morphological, molecular and chemical techniques to obtain information regarding of characterization, identification, discrimination and classification of *Passiflora* species. In this context, several analytical techniques have been employed in the chemical characterization and discrimination of medicinal plants, offering unique advantages and applications. These techniques comprise nuclear magnetic resonance (NMR), gas chromatography (GC), and high performance liquid chromatography (HPLC), both coupled to mass spectrometry (MS), as well as infrared (IR) and ultraviolet (UV) spectroscopies (Li et al. 2018; Liu et al. 2019; Biancolillo et al. 2020; Brahmi et al. 2020; Jamwal et al. 2020; Leyva et al. 2021; McCullagh et al. 2021; Wang et al. 2021; Dutra et al. 2023). Additionally, chemometric tools have become extremely important for investigate complex samples analyzed by spectroscopy techniques. The multivariate analysis providing the extraction of relevant information from datasets with extensive overlapping and large amount of information, such as those found in all analytical techniques. In this respect, chemometric methods applied in medicinal plant research include exploratory analysis of chemical data using hierarchical cluster analysis (HCA), principal component analysis (PCA), as well as multivariate calibration employing principal component regression (PCR), partial least-squares discriminant analysis (PLS-DA), k-nearest neighbors (KNN), soft independent modeling of class analogy (SIMCA) (Li et al. 2018; Liu et al. 2019; Biancolillo et al. 2020; Brahmi et al. 2020; Jamwal et al. 2020; Leyva et al. 2021; McCullagh et al. 2021; Wang et al. 2021; Dutra et al. 2023).

Furthermore, analytical techniques as gas chromatography coupled to mass spectrometry (GC-MS) and high performance liquid chromatography coupled to mass spectrometry (HPLC-MS) are commonly employed for quality control and distinction of medicinal plants (Li et al. 2018; Liu et al. 2019; Wang et al. 2020; Zhang et al. 2020). These technologies have great potential for accurate analyses or standardization of herbal products. In *Passiflora*, the application of NMR and MS technique tools combined with multivariate data analysis for metabolic profile and quality control purposes was examined by Farag et al. (2016). They investigated the metabolic fingerprint of *Passiflora* extracts of 17 accessions from different geographic locations. Additionally, the authors identified 78 metabolites (mainly flavonoids and fatty acid conjugates), and notably, *C*-flavonoids, including compounds like isovitexin-2″-*O*-xyloside, luteolin-*C*-deoxyhexoside-*O*-hexoside, schaftoside, isovitexin, and isoorientin, were significant contributors to the discrimination of *Passiflora* species (Farag et al. 2016). The author discovered that metabolites provided a comprehensive map of secondary metabolite distribution the *Passiflora* (Farag et al. 2016). In another study, High-Resolution Magic-Angle Spinning nuclear magnetic resonance spectroscopy (HR-MAS NMR) metabolic fingerprint analysis of seven *Passiflora* herbal medicines, confirmed plant species and identify biomarkers, like as vitexin, isovitexin and salicin (Flores et al. 2020). Recently, our research group reported that ^{1}H NMR-based metabolic fingerprint coupled with chemometric analysis can be employed to characterize extracts from wild *Passiflora* species (*P. alata*, *P. cincinnata*, and *P. setacea*) and their genetic varieties by comparing the chemical composition (Dutra et al. 2023). In our study, PCA allowed discrimination of *Passiflora* extracts, which the metabolites quadranguloside, oleanolic acid-3-sophoroside, α-glucose, β-glucose, and vitexin-2-*O*"-rhamnoside were relevant in the differentiation of *P. alata* and they genetic variety, while the varieties of *P. setacea*, and *P. cincinnata* were chemically equivalent to the original *Passiflora* species (Dutra et al. 2023). These works showed that the techniques can be efficient for differentiation, quality control and can be contribute to the evaluation of new tools to investigate *Passiflora* herbal medicines.

However, these tools possess great disadvantages, since considered extremely expensive, destructive, sample pretreatment requirements, and lengthy analysis times (Li et al. 2018; Wang et al. 2020). In contrast, vibrational spectroscopies have emerged as nondestructive, reagent-free, rapid, cost-effective that need little sample preparation alternatives for quality control

and discrimination of medicinal herbs (Wang et al. 2018; Biancolillo et al. 2020; Wang et al. 2021). Over the last decade, Attenuated Total Reflection Fourier transform infrared (ATR-FTIR) has become an important tool due to its ability to provide a comprehensive fingerprint in complex matrices, like medicinal plants. Compared to other vibrational techniques, ATR-FTIR offers advantages such as quickness, simplicity, excellent cost-effectiveness, non-invasive, and efficiency. It allows for the analysis of liquid, solid samples or maintained in their natural state, without need for sample pretreatment. In addition, it eliminates the use of KBr during for transmission analysis (Wang et al. 2018; Biancolillo et al. 2020). ATR-FTIR spectroscopy coupled with chemometrics has been largely used in quality assessment of herbal medicines (Li et al. 2018; Wang et al. 2018), differentiation of fungi, medicinal plants and foods (Rodríguez-Solana et al. 2014; Cortés et al. 2018; Biancolillo et al. 2020), as well as adulteration and taxonomical studies (Meinen and Rauber 2015; Rana et al. 2018; Jamwal et al. 2020). This technique effectively probes the chemical composition of matrices, considering the chemical composition of the species.

Until now, there are no publications related to the discrimination of *Passiflora* using ATR-FTIR combined with chemometric. In light of this, the aim of this work is to evaluate the potentiality of ATR-FTIR coupled to PCA for distinction purposes of *Passiflora* species. This research can contribute to quality control and providing insight for the investigation of new alternatives of herbal medicines based on *Passiflora*.

MATERIAL AND METHODS

Plant material

Leaves of six different *Passiflora* species, *P. alata* (Pa), *P. cincinnata* (Pc), and *P. setacea* (Ps), *P. alata* var. BRS Mel do Cerrado (PaBRSMC), *P. cincinnata* var. BRS Sertão Forte (PcBRSSF), and *P. setacea* var. BRS Pérola do Cerrado (PsBRSPC) were employed in the current study. The genetic varieties were collected and identified in November 2017, while the others samples were obtained in August 2019, all by the Brazilian Agricultural Research Corporation (EMBRAPA) Semiarid, in Petrolina, state of Pernambuco, Brazil. All *Passiflora* spp. were dried in an oven of circulating air at 45 °C for 72 h. Finally, each sample was submitted to pulverization in an analytical mill (Quimis, São Paulo, Brazil) and sieves for standardization of particle size (0.425 mm), before the freezing. Subsequently, the *Passiflora* spp. were kept at - 20 °C for further analysis. All procedures for access to genetic patrimony and associated traditional knowledge were carried out and the project was registered in SisGen (Register #A1514EF).

ATR-FTIR spectroscopy

ATR-FTIR spectra of *Passiflora* samples were obtained using an IRTracer-100 Fourier Transform Infrared Spectrophotometer (Shimadzu Corporation, Kyoto, Japan) equipped with a universal ATR composed of a diamond/ZnSe crystal. Absorbance spectra were in the wavenumber range from 4000 to 600 cm^{-1} (Mid IR Region), acquiring 64 successive accumulations per sample at a resolution of 4 cm^{-1}, and Happ-Genzel apodization function. The dried powder of *Passiflora* spp. (5 mg) was coated onto the crystal for the infrared spectroscopic analysis. After each measurement, the crystal was cleaned with a soft tissue soaked with methanol and left to dry in ambient air. A background spectrum of air was scanned under the same instrumental conditions before each series of measurements. The system was operated using the LabSolutions IR software (Shimadzu Corporation, Kyoto, Japan). Each sample was analyzed in 5 replicates amounting to 30 experiments. The room was maintained at ambient temperature (25 °C) and relative humidity (30%).

Pre-processing of the data

All ATR-FTIR spectra of *Passiflora* samples were arranged in the Origin Pro 8 software (OriginLab Corporation, Massachusetts, United States), which columns represent samples, while rows, the variables (absorption intensity). Subsequently, spectral data has been performed in MATLAB® software (Math Work Inc., Massachusetts, United States) using PLS-Toolbox 3.0 (Eigenvector Research) for further principal component analysis (PCA). The preprocessing of ATR-FTIR spectral data is crucial before conducting multivariate analysis, especially when dealing with potential issues like baseline drift, overlapping peaks, and sample particle size variations (Li et al. 2018). These preprocessing steps help to enhance the quality of the data and the effectiveness of subsequent analyses, such as PCA. Thus, ATR-FTIR spectra were processed using the combination of two pretreatment methods, by applying mean centering followed by detrend. The mean centering was chosen on data to remove similar characteristics in all spectra thereby emphasizing spectral variance (Beljebbar et al. 2010), while detrend function detects and eliminates linear trends in the data spectral (Semaan and Yadav 2020). Therefore, overall intensity offset and any linear trends from the spectra were removed, thus ensuring the spectral data were appropriately preprocessed for PCA.

Chemometric analysis

Principal components analysis (PCA) were performed in MATLAB® software (version 7.0.1, Math Work Inc., Massachusetts, United States) using PLS-Toolbox 3.0 (Eigenvector Research). PCA was employed as a data diminution where each spectrum, which consists of hundreds of absorbance values, is represented by a point in a multidimensional space using a linear transformation, to obtain qualitative information about the possible discrimination by exploration of the data structure and the relationship between variables (Beghi et al. 2017), as well as to identify possible outliers through the analysis of Hotelling's T^2 (see supplementary material). In this work, no outliers were detected. The wavenumbers between 4000 to 600 cm^{-1} represent all ATR-FTIR vibrational regions in the samples. The absorptions at 2600 to 2100 cm^{-1} were excluded from the ATR-FTIR data to eliminate CO_2 peaks and interferences in the spectra, respectively. After spectra pre-treatments, the initial matrix was constituted by 30 samples (lines) and 752 variables (columns). To visualize similarities and tendencies between samples, score plots was used, while loading plots reveal the contribution of the original variables, in this case, the wavenumbers that represent the functional groups typical of the compounds.

RESULTS AND DISCUSSION

ATR-FTIR fingerprint analysis

Passiflora leaves are complex samples recognized by the production of secondary metabolites, mainly flavonoids (Patel et al. 2011; Farag et al. 2016; Dutra et al. 2023). The IR spectroscopic absorption band can be attributed to biological compound classes, such as flavonoids, lignins, lipids, and polysaccharides and terpenoids (Kumar et al. 2015; Macedo et al. 2023; Pedrosa et al. 2024). The Figure 1 showed the ATR-FTIR spectra for the six *Passiflora* species. All ATR-FTIR fingerprint revealed similar absorption bands and positions in the spectra of *Passiflora*, but with the different absorption bands height (Figure 1). Thus, the infrared metabolic profiling results indicated that the spectra of *Passiflora* in all spectral range 4000 to 600 cm^{-1}, however, a large number of chemical absorption bands were verified in the region 2000 to 600 cm^{-1} with certain discrepancies (Figure 1).

In all spectra of *Passiflora* spp. were observed several absorbance bands in the spectral region around 1750 to 600 cm^{-1}, as well as in the spectral region from 3710 to 2800 cm^{-1}. The broad band centered at 3325 cm^{-1} was attributed to the –OH stretching vibrations, which are present in chemical metabolites, as phenolic compounds and polysaccharides (Mularczyk-Oliwa et al. 2012; Li et al. 2018; Wang et al. 2018; Agatonovik-Kustrin et al. 2020). Also, two bands at 2916 and 2850 cm^{-1} were assigned to the $–CH_3$ and $–CH_2$ stretching absorptions related to lignins, lipids and terpenoids (Li et al. 2018; Álvarez et al. 2020). The presence of absorptions at 880, 835, 775, 664, and 626 cm^{-1} were associated with vibrations of aromatic ring substitution patterns. The bands at 664 and 775 cm^{-1} actually results from =C–H out-of-plane bending vibrations of aromatic compounds, which the region around 880 to 835 cm^{-1} showed the existence of the =C–H out-of-plane bending, typical of the *para*-disubstituted ring. Furthermore, the absorptions at 880, 775, 664, and 626 cm^{-1} also can also reveal *ortho*- and *meta*-disubstituted systems (Chaudhari et al. 2015. Agatonovik-Kustrin et al. 2020). These vibrational absorptions are resulting from the strong coupling with adjacent hydrogen atoms. On the other hand, the sharp bands 1735, 1643, and 1546 cm^{-1} can be associated to band typical of the C=O stretching and aromatic ring (C=C) vibrations indicative of functional groups present in phenolic compounds, like polyphenol and flavonoids, as well as polyesters, pectins and lignins (Li et al. 2018; Álvarez et al. 2020; Agatonovik-Kustrin et al. 2020).

Moreover, the absorption bands at 1411 and 1377 cm^{-1} displayed the presence of –OH vibration corresponding to organic acid and lipids, whereas the absorptions at 1315 and 1230 cm^{-1} were awarded to C–O stretching vibrations indicatives of polysaccharides, pectin and lignins (Li et al. 2018; Agatonovik-Kustrin et al. 2020; Álvarez et al. 2020). Also, the peaks at 1107 and 1037 cm^{-1} were related to –OH deformations (Figure 1). Based on the available literature, phenolic compounds are very common in *Passiflora* species, mainly *O*- and *C*-flavonoid glycosides derived of the apigenin and luteolin, as vitexin, orientin, isovitexin, and isoorientin, and considered chemotaxonomic makers of various *Passiflora* (Patel et al. 2011; Gazola et al. 2018; McCullagh et al. 2021; Dutra et al. 2023). The fingerprint of the *Passiflora* species is very similar and possesses intense overlapping, which hamper the observation of the difference between the metabolites in the samples. In this way, chemometric analysis was performed to track the vibrational absorptions of compounds important for characterization, as well as the distinction of *Passiflora* species.

Principal component analysis of the ATR-FTIR fingerprint

In this work, PCA was performed on the ATR-FTIR data to investigate main differences or similarities in the *Passiflora* spp. and to highlight possible relevance among samples and metabolites. With a total of 30 samples, (6 with 5 replicates),

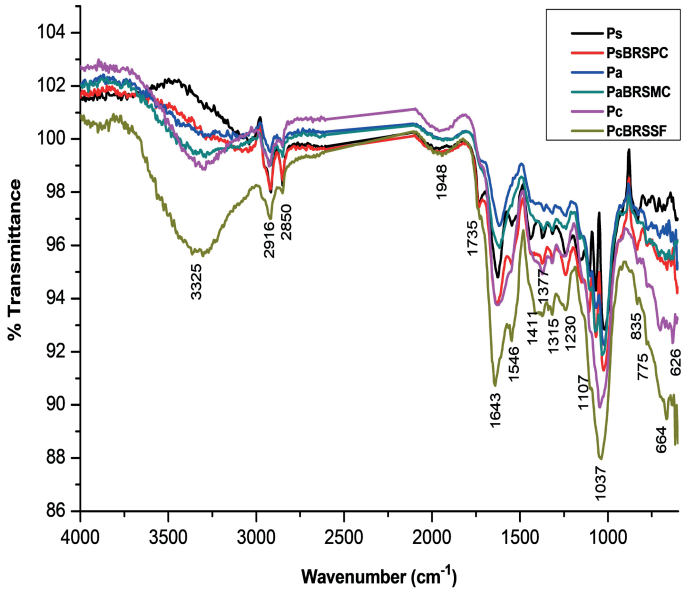

Figure 1. ATR-FTIR fingerprint of *Passiflora* species investigated in this study. - *P. alata* (Pa), - *P. alata* var. BRS Mel do Cerrado (PaBRSMC), - *P. cincinnata* (Pc), - *P. cincinnata* var. BRS Sertão Forte (PcBRSSF), - *P. setacea* (Ps), - *P. setacea* var. BRS Pérola do Cerrado (PsBRSPC).

chemometric results were significant for better representation of ATR-FTIR spectra. In this way, PCA was used to reduce the number of variable and early detection of sample grouping/discrimination of the *Passiflora* species. Scores plot obtained through the PCA analysis applied to the ATR spectra of the samples are depicted in Figure 2A. The first two principal components (PCs) were shown to carry the maximum variation among the data matrix explained 94.28%, which PC1 representing 84.86%, while PC2 demonstrated an additional of 9.42% (Figure 2A).

In the 2D-scores plot, a well-defined six groups were clearly observed. The first and second groups were constituted of the samples of Ps (black triangles) and PsBRSPC (red diamonds), on positive side of PC1 and negative side of PC2. On the other hand, Pa samples (blue squares) occupying the positive sides of PC1 and PC2, representing the third group, while the fourth group was composed of PaBRSMC (blue stars), which are distributed in the negative region of PC1 and positive region of PC2. The variety PcBRSSF (green dots) have samples with dispersion, although most are located on the negative sides of PC1 and PC2, however, Pc (pink crosses) is found in all quadrants of PCA, revealed the last two groups identified (Figure 2A). Although they occupy the same quadrant, there is a clear distance between *P. setacea* and their genetic variety, which suggests chemical differences between them. The investigation of the PC1 and PC2 loadings plot allowed the identification of variables with the highest influence on the data variance (Figures 2B and 2C). Thus, the loadings detail the wavenumbers responsible for the differentiation of the species, or whatever the values of the variables described wavenumbers related to the functional groups characteristic of the compounds present in the *Passiflora* samples (Figures 2B and 2C).

The loadings plot of PC1 and PC2 displayed four absorption range with positive side in PC1 (3649-3079 cm^{-1}, 1068-1002 cm^{-1}, 891-875 cm^{-1}, 705-632 cm^{-1}) and negative side in PC2 (3572-3236 cm^{-1}, 1049-1033 cm^{-1}, 898-883 cm^{-1}, 717-601 cm^{-1}), suggesting that absorption bands are responsible by the discrimination of Ps and their genetic variety (PsBRSPC) from other *Passiflora* species (Figure 2B and 2C). The wavenumbers at 891 to 875 cm^{-1}, 898 to 883 cm^{-1}, 705 to 632 cm^{-1}, and 717 to 601 cm^{-1} revealed the presence of =C-H and C=C out-of-plane bending associated to substituted aromatic rings, while the bands around 1068 to 1002 cm^{-1} and 1049 to1033 cm^{-1} are indicatives of C–O and C–C stretching vibrations. In turn, the absorption bands at 3649 to 3079 cm^{-1} and 3572 to 3236 cm^{-1} were attributed to the –OH stretching vibrations. In this regard, these wavenumbers range which is part of *Passiflora* fingerprints might be attributed to the presence of flavonoids, polysaccharides, and pectins (Figure 2A and 2B).

On the other hand, the absorption bands at negative sides of PC1 (2846-1720 cm^{-1} and 813-686 cm^{-1}) and PC2 (2808-1751 cm^{-1} and 813-798 cm^{-1}) were relevant in the distribution of Pc and PcBRSSF in the scores plot (Figure 2). The wavenumbers range at 2846 to 1720 cm^{-1} and 2808-1751 cm^{-1} are representative of $–CH_2$ and $–CH_3$ stretching bands,

as well as C=O stretching vibrations attributed to carbonyl compounds, whose absorptions were associated with terpenoids, lipids, lignins and flavonoids and revealed high significance for Pc and PcBRSSF species (Figure 2B and 2C). Moreover, the loadings plot exhibited vibrational absorptions in positive sides of PC1 (1662-1635 cm^{-1} and 1600-1581 cm^{-1}) and PC2 (1628 cm^{-1} and 1543 cm^{-1}) related to C=C stretching absorptions typical of the aromatic rings (Figure 2B and 2C), which were responsible for the discrimination of Pa. However, absorption bands identified in the region in negative side of PC1 (1519-1419 cm^{-1}) and positive side of PC2 (1489-1415 cm^{-1}) were strongly correlated with PaBRSMC (Figure 2B and 2C). These wavenumbers range were associated with C-O stretching and –OH bending vibrations indicative of characteristic functional groups of phenolic compounds, polyphenols and saponins (Li et al. 2018; Wang et al. 2018).

These observations revealed that flavonoids played a relevant role in the differentiation of *Passiflora*. This study can be corroborated considering previous works with *Passiflora* species that showed the identification of flavonoids isoorientin in PcBRSSF extract, while isovitexin and isovitexin-2''-*O*-xyloside were identified in the Pc, PcBRSSF, Ps and PsBRSPC extracts, in addition vitexin and vitexin-2''-*O*-ramnoside were observed in Pa and PaBRSMC extracts, as well as the saponins quadranguloside and oleanolic acid-3-sophoroside (Dutra et al. 2023). Furthermore, the flavonoid vitexin-2''-*O*-xyloside was identified only in Pc and PcBRSSF, whereas α-glucose, β-glucose, sucrose, trigonelline, saturated and unsaturated fatty acids were identified in all species investigated in the research (Dutra et al. 2023). This work also discusses a large number of the biological activities attributed to metabolites based on literature.

Thus, *Passiflora* species are a rich source of metabolites, mainly flavonoids, in addition to being responsible for several biological activities in the genus (Patel et al. 2011; Gazola et al. 2018; McCullagh et al. 2021; Dutra et al. 2023). In another research, fourteen secondary metabolites were identified in ethanolic extract of *P. cincinnata* investigated by HPLC-DAD-MS/MS, mainly *O*- and *C*-flavonoid glycosides from apigenin, orientin, isoorientin, vitexin, and isovitexin described in other Passifloraceae as previously reported in *P. alata* and *P. setacea* (Doyama et al. 2005; Patel et al. 2011; Gazola et al. 2018; Leal et al. 2020; Sanchez et al. 2020). Time of collection, edaphoclimatic aspects, as well as genotypic variations between species can influence the chemical profile of plant matrices (Dutra et al. 2023). Furthermore, the results demonstrated that differentiation of *Passiflora* species based on attenuated reflectance spectroscopy fingerprint can be effective even if investigated chemical profiles are very similar.

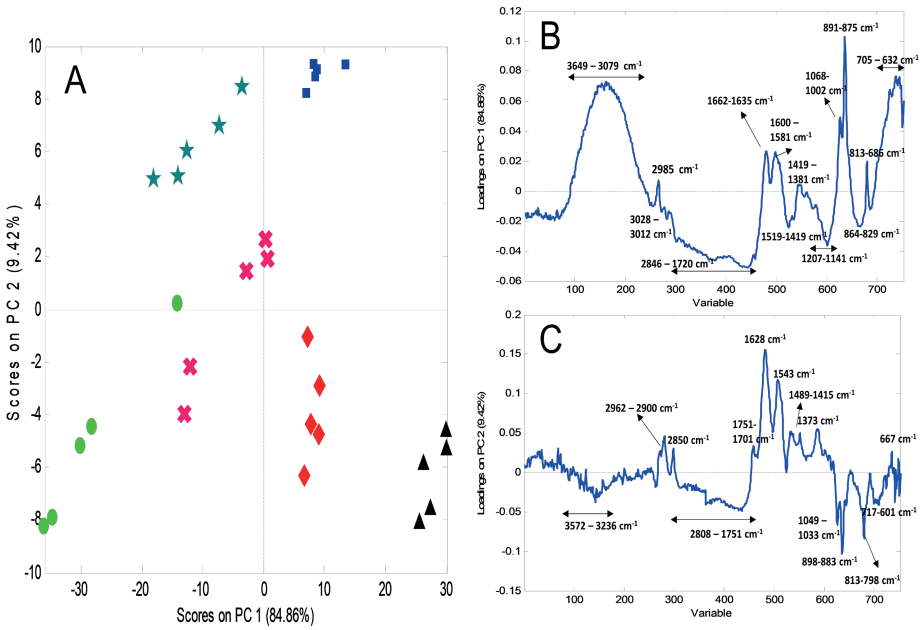

Figure 2. Principal components analysis (PCA) plot using ATR-FTIR spectra of *Passiflora* spp. (A) Scores plot of *Passiflora* species showing PC1 (84.86%) versus PC2 (9.42%), Ps (black triangles), PsBRSPC (red diamonds), Pa (blue squares), PaBRSMC (blue stars), Pc (pink crosses), and PcBRSSF (green dots). (B) Loadings plot of variables versus PC1 and (C) Loadings plot of variables versus PC2 are also depicted, discriminating the spectral range responsible for the separation of groups.

CONCLUSION

In conclusion, the ATR-FTIR spectroscopy combined with chemometric methods has potential to discriminate and characterize P*assiflora* species. Moreover, the chemical profile based on this spectroscopic tool displayed sharp and intense vibrational absorptions that agree to major functional groups, reducing time over data analyses. In comparison with other techniques, ATR-FTIR providing a simple, efficient, rapid, non-destructive screening method, as well as, no sample preparation, demonstrating that could be an alternative for the quality control and chemophenetic studies of medicinal plants based on the relevance of their functional groups, in the word, the chemical composition.

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AUTHORS' CONTRIBUTIONS

LMD: Experimental design, acquisition/ interpretation ATR-FTIR data, chemometric analysis, manuscript writing, edition and revision. PHVT: manuscript writing and revision. NN: Chemometric analysis, interpretation of the data and manuscript revision. NFM: Plant collection, taxonomical confirmation and manuscript revision. JRGSA: leader of the group, experimental design, interpretation of the data, critical reading of the manuscript and revision. All authors have read the final manuscript and approved the submission.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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