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Dissertação apresentada ao Instituto de Biociência da Universidade de São Paulo, para a obtenção de Título de Mestre em Ciências, na área de Botânica.

Orientadora: Dra Déborah Yara Alves Cursino dos Santos

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Profa Dra Déborah Yara Alves Cursino dos Santos

Orientadora

"And now here is my secret, a very simple secret: It is only with the heart that one can see rightly; what is essential is invisible to the eye".

The Little Prince - Antoine de Saint Exupéry

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Chapter 1. Secondary metabolites and biological activities of *Feijoa sellowiana* (synonym *Acca sellowiana*) - a review

Abstract

Feijoa (*Feijoa sellowiana*, synonym *Acca sellowiana*) is a South American shrub, known mainly for the edible fruits, which present pleasant aroma and flavor responsible for making it a popular ingredient in a variety of food products. However, there is a wealth and chemical diversity of secondary metabolites not only in fruits, but also in the other parts of the plant. The purpose of this review is to compile reported information related to the chemistry of feijoa, aiming expand the knowledge and aggregate value to this species. Based on the literature, this review focuses on the volatile and phenolic compounds that have been reported for this species, including information concerning the various extraction and analytical methods used, and mentioning some biological activities in which extracts and/or isolated compounds have shown good results. The volatile oil of feijoa has been analyzed mainly in fruits and leaves, obtained by headspace solid-phase microextraction (HS-SPME), solvent-assisted extraction (SAFE), and hydrodistillation, and analyzed trough gas chromatography coupled with mass spectrometry (GC-MS). A wide range of volatiles has been identified in fruits, including esters, terpenes, aldehydes, ketones, and alcohols, while terpenes were predominant in leaves. Furthermore, feijoa contains a rich variety of phenolic compounds such as phenolic acids, as well as flavones and other flavonoids. Conventional extraction methods (e.g. maceration) and innovative methods (e.g. ultrasound assisted extraction) have been widely employed. Antioxidant, antimicrobial, anti-inflammatory, anticoagulant, enzyme inhibitors, and antiproliferative effects have been

associated with compounds present in feijoa extracts. Concluding, this review opens new insights of possible applications in food, pharmaceutical, and cosmetic industries.

1. Introduction

Feijoa (*Feijoa sellowiana* (O. Berg) Burret, syn A*cca sellowiana*) is a perennial shrub (two to four meters height) native to the subtropical zone of South America, belonging to Myrtaceae, with scaly brownish-red bark, simple, opposite, elliptical, and pubescent leaves, bisexual and solitary flowers with four petals and numerous stamens with reddish filaments. The fruit is a berry measuring two to six centimeters in length (Cabrera et al., 2018), weighing between 40 and 50 grams. It has a sweet and sour taste. The epicarp is dark green and may be slightly wrinkled (Flor de planta, 2017). The pulp has a clear gelatinous portion that surrounds the seeds, and a firmer, slightly granular, opaque, and fleshy part (Flor de planta, 2017; Amaral et al., 2019; Phan et al., 2019).

Feijoa is widely cultivated due to its tasty and aromatic fruits, which are used in the production of numerous food products such as juices, jams, yogurts, among others (Phan et al., 2019). In addition to the culinary use, feijoa is also used in some natural cosmetic products (Zhu et al., 2018).

Research on feijoa cultivation and its bioactive components has been conducted in various countries, including Brazil, Italy, Japan, Morocco, Turkey, Iran, Egypt, Czech Republic, New Zealand, and Colombia. These studies aim to explore the potential health benefits and applications of this species in various industries, including food, pharmaceutical, and cosmetic.

The volatile oils of feijoa are characterized by the presence of terpenes, esters, alcohols, and ketones. Terpenes and esters are responsible for its characteristic aroma. Some notable examples include linalool, methyl, and ethyl benzoate, (Z)-3-hexenyl, limonene, β-caryophyllene, aromadendrene, and α-copaene (Shaw et al., 1989; Mosbah et al., 2018; Peng et al., 2020; Cebi & Sagdic, 2022).

Furthermore, feijoa contains a rich variety of phenolic compounds. Phenolic acids such as *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, ellagic acid, and gallic acid have been described, as well as flavones and other flavonoids including rutin, quercetin, floretin, and naringenin (Sun-Waterhouse et al., 2013; Aoyama et al., 2018; Santos et al., 2019; Cebi & Sagdic, 2022).

Flavones have demonstrated antibacterial and antifungal properties (Basile et al., 2010). Ellagitannins, such as pedunculagin, have been associated with tyrosinase inhibitory activity and have shown antitumor effects, while compounds such as polyphenols, flavonoids, and ascorbic acid contribute to its antioxidant properties (Aoyama et al., 2018). Finally, anticholinesterase activity has been observed, which may be related to the abundance of quercetin derivatives in the leaves.

In this review, we summarize and compare the main extraction methods used and the secondary metabolites identified within leaves, fruits, stems and flowers of *Feijoa sellowiana* (also known as *Acca sellowiana*). We also highlight the bioactivities of isolated compounds from this species and make

recommendations for future studies. Relevant literature was obtained by searching ‘Feijoa sellowiana’, ‘Acca sellowiana’, ‘secondary metabolites’ and ‘bioactive compounds’ into Google Scholar, ScienceDirect, PubMed and Scopus databases. In some cases, specifying secondary metabolites as 'phenolic’ or ‘volatile compounds’ was necessary to make the search more accurate.

2. Results and discussion

2.1 Volatile compounds

Phytochemical research on volatile compounds in feijoa reveals a diverse range of secondary metabolites that contribute to the characteristic aroma and flavor of the plant. These studies, employing various analytical techniques and extraction methodologies, shed light on the complex chemical composition of this species and its potential applications in various fields.

The most used extraction methods were headspace solid-phase microextraction (HS-SPME), solvent-assisted extraction (SAFE), and hydrodistillation (Table 1). These techniques differ in selectivity and efficiency in capturing volatile compounds, which impacts the detection and quantification of specific components. Gas chromatography coupled with mass spectrometry was the analytical technique used for identification. Hydrodistillation is the conventional method for extracting essential oils, using steam to carry volatile compounds. Although this method is notable for not generating chemical contaminants, its limitations include the need for large sample quantities, slow process, and potential degradation of certain thermosensitive compounds (Fagbemi et al., 2021). On the other hand, SAFE extraction has been widely applied to isolate aromatic compounds from complex matrices, mainly in food.

This method is effective at low temperatures, thus preserving heat-sensitive compounds (Xu et al., 2007; Huang et al., 2019). Lastly, HS-SPME stands out for its selectivity and sensitivity in absorbing volatile compounds onto a fiber. It is a fast, cost-effective method requiring small sample amounts and no solvents (Sporkert et al., 2000).

Table 1. Compilation of studies on volatile compounds of *Feijoa sellowiana* highlighting the plant part used, the extraction method, the main compounds found and the country of the sample origin.

Plant Part	Extraction Method	Main Compounds	Country	Reference
Fruit (skin)	Hydrodistillation (Clevenger-type apparatus)	β-cariofileno (12%), ledeno (9.6%), a-humuleno (6.3%), β-elemeno (4.9%) y 8-cadineno (4.8%).	France	Fernandez et al., 2004
	Hydrodistillation (Clevenger-type apparatus)	Caryophyllene was the most abundant 17.7%, followed by Germacrene D 14.4%, Humulene 10.5%, Ledene 14%, Spathulenol 8.5%, C	Moroccan	Elfarnini et al., 2018
Fruit (pulp)	Mixture of dichloromethane/n-pentane (100 ml, 1:1, v/v). Solvent-assisted flavor evaporation (SAFE)	Ethyl butanoate, Z-3-hexenal, linalool, and methyl benzoate	Colombia	Sinuco et al., 2020
	Headspace solid-phase microextraction coupled to GC-MS (HS-SPME–GC–MS)	(Z)-3-Hexen-1-ol, linalool, and methyl benzoate constituted 53% of the oil	New Zealand	Shaw et al., 1989
	Hydrodistillation	γ-Selinene (17.39%), α-Caryophyllene (16.74%), β-Caryophyllene (10.37%), and Germacrene D (5.32%)	Italy	Smeriglio et al., 2019
Fruit (whole)	HS-SPME–GC–MS	(Z)-3-hexen-1-ol, ethyl butyrate, methyl benzoate, ethyl benzoate, (E)-β-ocimene, (Z)-3-hexenyl acetate, octan-3-one, and linalool	New Zealand	Zhao et al., 2023
	HS-SPME–GC–MS	Methyl, ethyl, and (Z)-3-hexenyl benzoate with 50% of the volatile composition	Colombia	Baena-Pedroza et al., 2020

	HS-SPME–GC–MS	Methyl benzoate as the dominant compound. Ester and terpene were the dominant groups	New Zealand	Peng et al., 2020
Leaves	HS-SPME–GC–MS	Limonene (36.2%), β-Caryophyllene (27.8%), aromadendrene (12.5%), and α-Copaene (6.6%)	Turkey	Mosbah et al., 2018
	HS-SPME–GC–MS	Limonene (33.46%), linalool (18.95%), and caryophyllene (22.18%)	Turkey	Cebi & Sagdic, 2022
	Not reported	Caryophyllene oxide (24.3%), linalool (7.9%), and spatulenol (6.6%)	Egypt	El-Nashar et al., 2022
	HS-SPME–GC–MS	(Z)-3-hexen-1-ol, ethyl butyrate, methyl benzoate, ethyl benzoate, (E)-β-ocimene, (Z)-3-hexenyl acetate, octan-3-one, and linalool	New Zealand	Zhao et al., 2023
Stem	Not reported	Caryophyllene oxide (38.1%), α-zingiberene (10.1%), and humulene II (6.0%)	Egypt	El-Nashar et al., 2022

The most used parts of the plant for volatile compound evaluation are leaves and fruits (Table 1). The chemical profile significantly varied between them. In fruits, a wide range of secondary metabolites has been identified, including esters, terpenes, aldehydes, ketones, and alcohols. Esters like methyl benzoate, ethyl benzoate, along with alcohols like (Z)-3-hexenyl, were the most abundant compounds in most studies using either whole fruit, pulp, or peel. These compounds stand out for conferring the characteristic fruity and floral aroma to the fruit (Baena-Pedroza et al., 2020). On the other hand, terpenes were generally the most diverse group of compounds in leaves. Regarding other parts of the plant, El-Nashar (2022) identified three sesquiterpenes in the stem. Volatile compounds have not been studied using flowers or seeds.

Geographical variation in volatile compound profiles reflects the plant's adaptation to different environments. Differences in chemical composition could be related to climatic conditions, genetic factors, nutrient availability, and other environmental factors. These results underline the influence of the environment on secondary metabolite production and emphasize the importance of considering geographical location when interpreting aromatic profile differences (Sampaio et al., 2016). The data in the Table 1 highlight the complexity and diversity of volatile compounds present in this species, providing relevant information for understanding its chemical composition and potential applications in the food, cosmetic, and pharmaceutical industries.

2.2 Phenolics compounds

Numerous studies have provided a detailed and significant insight into the diversity and abundance of phenolic metabolites in various parts of feijoa, followed by potential biological activities linked to these compounds. It is worth

noting that extraction methods play a crucial role in obtaining these metabolites, and their choice can impact the results obtained. Both conventional extraction methods and innovative methods have been widely employed in studies focused on this species (Table 2).

Table 2. Compilation of studies on phenolic compounds of *Feijoa sellowiana*, highlighting the plant part used, the extraction method, the main compounds found, biological activities identified and the country of the sample origin.

Plant Part	Extraction Method	Main Compounds	Biological Activity	Country	Reference
Floral Buds	Maceration with MeOH	Cyanidin glucoside, ellagic acid, edunculagin, flavone, gallic acid and gossypetin arabinofuranoside	Tyrosinase inhibitor, antitumoral	Japan	Aoyama et al., 2018
Flowers	Maceration using an ultrasonic bath (3 cycles of 30 min at 10 ± 2 °C) with a hydroethanolic solution (EtOH:H_2O - 80:20 v/v), solvent:plant ratio 9:1 v/w	Cyanidin-3-O-glucoside, ellagic acid, pedunculagin and quercetin-3-O-galactoside	Antioxidant	Italy	Montoro et al., 2020
Fruit (peel)	Maceration in 80% methanol with 1% (v/v) HCl for 15 minutes in darkness at room temperature with agitation. Then, ultrasonication for 15 minutes, followed by centrifugation.	Catechin, ellagic acid and dihydroxyflavone	Antioxidant, antibacterial	Australia	Phan et al., 2019
	Soxhlet extraction using a mixture of ethanol-water (50:50 v/v) Ultrasound assisted extraction (UAE) at 250 W and 10 min with 250 W and 10 min Pressurized Liquid Extraction (PLE) at 100 bar and 40 °C using ethanol as the solvent	Ellagic acid, ferulic acid and gallic acid	Antioxidant, antibacterial	Brazil	Santos et al., 2019

	Supercritical Fluid Extraction (SFE): Temperature ranged from 40 to 55 °C, pressure from 200 to 300 bar. CO_2 flow rate: 8.33 g/min, extraction duration: 210 min. Ethanol as a cosolvent at 5% (w/w).				
	Accelerated solvent extraction (ASE), with water, acidified water, or various aqueous ethanol solutions (ethanol/water ratios of 30:70, 50:50, or 80:20) at either 20 °C or 50 °C.	2,4-dihydroxybenzoic acid, caffeic acid, catechin, chlorogenic acid, epicatechin, ferulic acid, m-coumaric acid, naringenin, p-coumaric acid, p-hydroxybenzoic acid, phloretin, phlorizin, procyanidin B1 dimer, protocatechuic acid and quercetin	Antioxidant	New Zealand	Sun-Waterhouse et al., 2013
	Optimal conditions: Solvent-to-sample ratio of 60:1, temperature set at 40 °C, and extraction duration of 3 hours employing aqueous acetone (80%).	Ferulic acid, o-coumaric acid, syringic acid and trans-cinnamic acids	Antioxidant	Turkey	Tuncel & Yılmaz, 2015
	Maceration with methanol 90 % l at room temperature for 18h on a shaker equipment	Procyanidin B-type dimer, procyanidin B-type tetramer and procyanidin C-type trimer	None	New Zealand	Zhao et al., 2023
Fruit (pulp)	Maceration in 80% methanol with 1% (v/v) HCl for 15 minutes in darkness at room temperature with agitation. Then, ultrasonication for 15 minutes, followed by centrifugation.	Catechin, dihydroxyflavone and ellagic acid	Antioxidant, Antibacterial	Australia	Phan et al., 2019

	Optimal conditions: Solvent-to-sample ratio of 60:1, temperature set at 40 °C, and extraction duration of 3 hours employing aqueous acetone (80%).	Syringic acid and trans-cinnamic acid	Antioxidant	Turkey	Tuncel & Yılmaz, 2015
	Maceration with methanol 90 % l at room temperature for 18h on a shaker equipment	Procyanidin B-type dimer, procyanidin B-type tetramer and procyanidin C-type trimer	None	New Zealand	Zhao et al., 2023
	Maceration with 70% acetone followed by extraction with ethyl acetate and n-butanol	4-O-α-arabinofuranosylellagic acid, flavone, gallic acid, isoquercitrin, pedunculagin and quercetin	Tyrosinase inhibitor, Antitumoral	Japan	Aoyama et al., 2018
	Maceration for 3 days with acetone at room temperature	Flavone	Antibacterial, Antifungal	Italy	Basile et al., 2010
	Soxhlet extraction using hexane and acetone with increasing polarity	Hexane: coumarin, steroid Acetone: coumarin, tannin, terpene	Antibacterial, Antifungal	Morocco	Elfarnini et al., 2018
	Fruit juice (UltraTurrax), followed by centrifugation 20 minutes at 15°C	Catechin, ellagic acid, eriocitrin, eriodictyol, gallic acid, pyrocatechol, quercetin, rutin and syringic acid	Anti-inflammatory, Antioxidant	Italy	Monforte et al., 2014
	Exhaustive extraction with methanol–distilled water (80:20, v/v)	Pedunculagin	Antioxidant	Brazil	De Oliveira Schmidt et al., 2020

	Fruit juice obtained with squeezer	(+)-catechin and procyanidin B1	Antioxidant	New Zealand	Peng et al., 2020
Fruit (whole)	Maceration in 80% methanol with 1% (v/v) HCl for 15 minutes in darkness at room temperature with agitation. Then, ultrasonication for 15 minutes, followed by centrifugation.	Catechin, dihydroxyflavone and ellagic acid	Antioxidant, Antibacterial	Australia	Phan et al., 2019
Leaves	Maceration with boiled ethanol 70%	Caffeic acid and gallic acid	Antioxidant, Anticoagulant, Antimicrobial	Egypt	Amer, et al., 2023
	Maceration with 70% acetone followed by extraction with ethyl acetate and n-butanol	Ellagic acid, flavone, gallic acid, hyperoside, pedunculagin and quercetin glucosides	Tyrosinase inhibitor, Antitumoral	Japan	Aoyama et al., 2018
	Supercritical Carbon Dioxide Extraction, ethanol as modifier (cosolvent)	Apigenin, catechin, ferulic acid, gallic acid, quercetin and rutin	Antioxidant	Iran	Bimakr et al. 2019
	Optimized extraction conditions (temperature (°C), time (min), feijoa concentration (g/mL), and shaking style (horizontal or vertical), using aqueous solutions	Caffeic acid, catechin, chrysin, ellagic acid, ferulic acid, gallic acid, p-Coumaric acid, quercetin and syringic acid	Antimicrobial	Turkey	Cebi & Sagdic, 2022
	Soxhlet extraction using hexane and acetone with increasing polarity	Hexane: Saponins, steroids, tannins, and terpenes Acetone: Coumarins, steroids and terpenes,	Antibacterial, Antifungal	Morocco	Elfarnini et al., 2018

	Maceration with Methanol/Water Extraction (7:3 v/v)	Isoflavonoids	None	Czech Republic	Lapcık et al., 2005
	Supercritical carbon dioxide extraction ($SC\text{-}CO_2$): Extractions with different levels of pressure (150-350 bar), temperature (40-60 °C) and dynamic extraction time (60-120 min). Maceration with ethanol (99.5% for 6 h (conventional extraction – CE)	$SC\text{-}CO_2$: Apigenin, catechin, ferulic acid, gallic acid, quercetin and rutin CE: Ferulic acid and gallic acid,	Antioxidant	Iran	Mousavi et al., 2018
	Ultrasound-assisted extraction process with ethanol	Apigenin, catechin, ferulic acid, gallic acid (most abundant), and quercetin	Antioxidant	Iran	Poodi et al., 2018
	Soxhlet extraction using a mixture of dichloromethane:methanol (80:20 v/v)	Avicularin, flavone (most abundant), primetin, quercetin and α-tocopherol	Antioxidant, Enzyme inhibition against α-glucosidase, amylase, tyrosinase, acetylcholine sterase, and butyrylcholin esterase	Egypt	Saber et al., 2021
	Maceration with methanol 90 % l at room temperature for 18h on a shaker equipment	Procyanidin B-type dimer, procyanidin B-type tetramer and procyanidin C-type trimer	None	New Zealand	Zhao et al., 2023

Petals	Maceration extraction using an ultrasonic bath (3 cycles of 30 min at 10 ± 2 °C) with a hydroethanolic solution (EtOH:H2O - 80:20 v/v), solvent:plant ratio 9:1 v/w	Cyanidin-3-O-glycoside, ellagic acid and Kaempferol-3-O-hexoside	Antioxidant	Italy	Montoro et al., 2020
	Juice Extraction (manual press)	Apigenin, cyanidin-3-O-glycoside, ellagic acid and Kaempferol-3-O-hexoside	Antioxidant	Italy	Montoro et al., 2020
Seeds	Maceration with methanol 90 % l at room temperature for 18h on a shaker equipment	Procyanidin B-type dimer, procyanidin B-type tetramer and procyanidin C-type trimer	None	New Zealand	Zhao et al., 2023
Stems	Not reported	Alkylated ellagic acids	Tyrosinase inhibitor, Antitumoral	Japan	Aoyama et al., 2018

The extraction process of phenolic compounds from feijoa has been addressed through a variety of methods, where solvent selection and manipulation of variables such as temperature are essential to maximize yields and preserve the integrity of heat-sensitive compounds. Individual solvents, often at specific concentrations, and solvent mixtures with different polarities have been the predominant approaches in these extractions, using simple cold or heated maceration for some hours. Such traditional methods have limitations in terms of yield, solvent consumption, duration, and waste generation, although they remain economical and easy to use.

However, there are already alternative methods aiming to address the disadvantages of conventional methods, including Microwave-Assisted Extraction (MAE), Supercritical CO2 Extraction (SC-CO2), Ultrasound-Assisted Extraction (UAE), Enzyme-Assisted Extraction (EAE), and Pressurized Fluid Extraction (PFE), even in strategic combinations. Some of these methods have already been used in studies of phenolic compounds of Feijoa sellowiana (Table 2). MAE uses microwave radiation to heat solvents and samples, expediting extraction, especially beneficial for obtaining short-chain phenolic acids and flavonoids. However, its use is restricted to solvents compatible with this type of radiation, and additionally, there is a risk of affecting thermosensitive compounds. On the other hand, SC-CO2 stands out for its selectivity and preservation of heat-labile compounds, allowing temperature and pressure adjustment. There is high efficiency in CO2 penetration into the sample, making it an ideal method for non-polar compounds. UAE, based on cavitation, enhances compound release by breaking

cell structures. This method is efficient, simple, requires a small solvent volume, and has a short extraction time. EAE employs enzymes for compound release, but it can be slower and more expensive due to enzymatic specificity. PFE operates with organic or aqueous solvents at high temperatures and pressure, excelling in efficiency and selectivity, but it can degrade thermosensitive compounds and be costly. These approaches enrich extraction options, offering specific advantages and limitations to strategically address challenges in obtaining phenolic compounds (Alara et al., 2021).

In general terms, comparing the chemical profiles of different parts of feijoa reveals remarkable variability in the composition of phenolic compounds. This chemical diversity can be attributed to factors such as geographical location, the specific biological function of each part, and the extraction methods employed. Flowers and fruits, particularly the peel, are rich in anthocyanins, flavonols, and ellagitannins, while leaves present a variety of phenolic acids and flavonoids (Table 2).

Regarding total phenolic content (TPC), variations associated with different parts of the plant, geographical location, and extraction methods are observed, as detailed in Table 3. Depending on the extraction method and solvents, the TPC could be different even using the same plant part, which means that the choice of extraction method and solvents plays a crucial role in the results. In the case of feijoa, the TPC quantification in all studies available was performed through the Folin–Ciocalteu assay, a spectrophotometric method based on an oxidation-reduction reaction, and gallic acid was used as a reference standard, which allowed the results to be expressed as gallic acid equivalents (GAE).

Despite these discrepancies, leaves and fruit consistently exhibit the highest values of total phenolic compounds compared to other parts of the plant, such as flowers and petals. In the specific case of the fruit, the peel shows the highest TPC content. It is important to note that a higher total phenolic content correlates positively with a greater antioxidant capacity (Song et al., 2010).

Table 3. Compilation of total phenol content (TPC) values reported for *Feijoa sellowiana*, highlighting the plant part used, extraction method, TPC value and country of origin of the sample.

Plant part	Extraction Method	Total phenolic compounds (TPC)	Country	Reference
Flowers	Ultrasound-assisted extraction (UAE) using 40% and 60% ethanol-water solutions Supercritical fluid extraction (SFE) Subcritical water extraction (SWE) Deep eutectic solvents (DES)	UAE: 5320–65,56 mg GAE/g dm* SWE: 42,88 and 58,39 mg GAE/g dm* SFE: 2,87–3,90 mg GAE/g dms* DES: 25,93 – 51,51 mg GAE/g dm*	Italy	Gil et al., 2023
	Maceration extraction using an ultrasonic bath (3 cycles of 30 min at 10 ± 2 °C) with a hydroethanolic solution (EtOH:H_2O - 80:20 v/v), solvent:plant ratio 9:1 v/w	395,14 ± 7.91 mg GAE/L	Italy	Montoro et al., 2020
Fruit (peel)	Solid-liquid extraction using a orbital shaker at 120 rpm for 160 minutes at 30 °C with 50% (v/v) aqueous ethanol	3,990.02 ± 22,40 mg GAE/100 g Fresh weight (FW)	Colombia	Burbano-Ipiales et al., 2022
	Maceration with acetone/water (70/30), followed by agitation for 1 hour at 19°C±1°C, finished with homogenization using Ultra-Turrax for 5 minutes at 0°C.	473,3 ± 0,0 mg GAE/100 g FW	Colombia	Sánchez-Riaño et al., 2020
	Extraction at room temperature by the percolation method using water or methanol	89,07 ± 0,54 mg GAE/g extract powder 69,14 ± 0,39 mg GAE/g extract powder	Iran	Ebrahimzadeh et al., 2008
	Maceration with methanol or 50% water for 1 hour	109,9 y 114,9 mg/100 g (FW)	Brazil	Amarante et al., 2017

	Ultrasound-assisted extraction (UAE) at 20 kHz for 20 min or shaking water bath extraction 24 h at 40 °C. Solvents (ethanol, acetone and distilled water) and concentration (2.5% and 5%).	349,17 – 517,19 mg GAE/100 g extract	Turkey	Karslı et al., 2021
Fruit (pulp)	Maceration with acetone/water (70/30), followed by agitation for 1 hour at 19°C±1°C, finished with homogenization using Ultra-Turrax for 5 minutes at 0°C.	1614,1 ± 16 mg GAE/100 g FW	Colombia	Sánchez-Riaño et al., 2020
	Maceration with methanol or 50% water for 1 hour	76,4 y 88,3 mg GAE/100 g FW	Brazil	Amarante et al., 2017
	Maceration with water/methanol 2:8 containing 2 mM NaF	92,88 ± 6,68 mg GAE/100 g FW (Smith) to 251,02 ± 25,87 mg GAE/100 g FW (Apollo)	Italy	Pasquariello et al. 2015
	Ultrasound-assisted extraction (UAE) at 20 kHz for 20 min or shaking water bath extraction 24 h at 40 °C. Solvents (ethanol, acetone and distilled water) and concentration (2.5% and 5%).	115,64 – 345,46 mg GAE/100 g extract	Turkey	Karslı et al., 2021
Fruit (Pulp +seeds)	Maceration with acetone/water (70/30), followed by agitation for 1 hour at 19°C±1°C, finished with homogenization using Ultra-Turrax for 5 minutes at 0°C.	595,9 ± 10,3 mg GAE/100 g FW	Colombia	Sánchez-Riaño et al., 2020
Fruit (whole)	Fruit juice obtained with squeezer	1,17 ± 0,01 mg GAE/mL juice (Opal Star) to 1,89 ± 0,01 mg GAE/mL juice (Wiki tu)	New Zealand	Peng et al., 2020
	Maceration with methanol	Fresh: 17,68 µg GAE/mg extract Dry: 8,69 µg GAE/mg extract	Turkey	Beyhan et al., 2010

	ultrasound-assisted extraction and ultrasound-assisted extraction coupled to pressurized liquid	27,8 to 43,4 mg GAE/g dry fruits	Brazil	Gambin et al., 2023
Leaves	Extraction at room temperature by the percolation method using water or methanol	92,09 ± 0,75 mg GAE/ g extract powder 44,17 ± 0,28 mg GAE/ g extract powder	Iran	Ebrahimzadeh et al., 2008
	Maceration with methanol	68,69 µg GAE/mg extract	Turkey	Beyhan et al., 2010
	Maceration with ethanol	179,43 ± 1,59 mg GAE/g extract	Tunisia	Mosbah et al., 2018
	Soxhlet extraction using methylene chloride: methanol mixture (80:20 v/v)	128,87 ± 0,92 mg GAE/g extract	Egypt	Saber et al., 2021
	Not reported	9,48 ± 0,02 mg/g extract	Tunisia	Mosbah et al., 2019
	Ultrasound-assisted extraction (UAE) at 20 kHz for 20 min or shaking water bath extraction 24 h at 40 °C. Solvents (ethanol, acetone and distilled water) and concentration (2.5% and 5%).	459,44 – 554,00 mg GAE/100 g extract	Turkey	Karslı et al., 2021
Petals	Maceration extraction using an ultrasonic bath (3 cycles of 30 min at 10 ± 2 °C) with a hydroethanolic solution (EtOH:H2O - 80:20 v/v), solvent:plant ratio 9:1 v/w	98,59 ± 7,89 mg GAE/L	Italy	Montoro et al., 2020
	Juice Extraction (manual press)	114,53 ± 9.46 mg GAE/L	Italy	Montoro et al., 2020

*Milligrams of gallic acid equivalent (GAE) per gram of residue (mg GAE/g dm)

2.3 Biological activities

Several biological activities such as antioxidant, antimicrobial, anti-inflammatory, anticoagulant, enzyme inhibitors, and antiproliferative effects have been associated with compounds present in feijoa extracts.

Antioxidant Activity

Various studies have found that compounds such as polyphenols, vitamin C, and flavonoids like quercetin are linked to antioxidant activity (Vuotto et al., 2000; Ebrahimzadeh et al., 2008; Zhu, 2018; Sánchez-Riaño et al., 2020). A study by Smeriglio et al. (2019) discovered that feijoa essential oil had the ability to neutralize various types of free radicals, along with a strong iron-chelating activity, which was also evidenced by Ebrahimzadeh et al. (2008) in the aqueous extract of *F. sellowiana* leaves.

Antimicrobial Activity

Studies conducted with feijoa leaf and fruit extracts have shown antibacterial and antifungal activity. The essential oil has a broad spectrum, especially against fungal strains, due to the presence of compounds such as limonene, β-caryophyllene, α-pinene, β-pinene, and terpinol (Saj et al., 2008). Fractions obtained from bark extracts exhibited antibacterial properties against *Staphylococcus epidermis, Escherichia coli*, and *Pseudomonas aeruginosa*, as well as antifungal activity against *Candida albicans* and *C. glabrata* (Motohashi et al., 2000). Other studies verified the strong *in vitro* antimicrobial activity of acetonic extracts from the fruit peel, suggesting flavones as the main compound responsible for this action (Motohashi et al., 2000; Phan et al., 2019). Basile (1997) described the antimicrobial activity of extracts from different

parts of the plant. The extracts were evaluated against eight bacterial strains, with seed extracts being the most efficient, followed by fruits and vegetative parts. The observed activity was exclusively bacteriostatic (Basile et al., 2010). The minimum inhibition concentrations (MICs) of leaf extracts against different bacterial and fungal strains ranged from 0.4 to 1.6 mg/mL and from 3.2 to 6.3 mg/mL, respectively (Mosbah et al., 2018). Zhu (2018) suggests that these antimicrobial properties may be related to the presence of bioactive compounds like polyphenols, including syringic acid and ellagitannins. An additional study by Vuotton (2000) using aqueous fruit pulp extracts showed inhibition against ten bacterial strains (MIC ranging from 1 to 64 mg/L). Gram-positive bacteria, *Streptococcus faecalis* and *Staphylococcus aureus*, were less sensitive to the extract and exhibited the highest MIC (MIC = 32 and 64 mg/L, respectively). Gram-negative bacteria such as *Enterobacter aerogenes* and *E. cloacae* (MIC = 2 mg/L) and *Pseudomonas aeruginosa* (MIC = 1 mg/L) showed the highest sensitivity to the extract. Higher sensitivity of gram-negative bacteria than gram-positive has already been described for the hydroalcoholic extract of *Syzygium cumini* (Myrtaceae) (Oliveira et al., 2007).

Antiproliferative Activity

The ability of feijoa acetone extracts to inhibit the growth of cancer cell lines has been documented, with flavones identified as the main contributor to this activity (Cassady et al., 1990; Bontempo et al., 2007). However, the evaluation of the antiproliferative capacity of purified flavone revealed that, although apoptotic action was detected, the effects on the cell cycle and differentiation were different from those observed with the crude extract, suggesting the presence of other components in the extract contributing to these activities (Bontempo et al., 2007). Elagitannins and other polyphenols identified

in feijoa leaves have inhibited the growth of certain types of cancerous tumors (Okuda et al., 1980; Amakura et al., 2000). Additionally, the proanthocyanidins described in feijoa demonstrated a cytotoxic effect against human oral squamous cell carcinoma cell lines (Aoyama et al., 2018).

Enzyme Inhibitory Activity

In the study conducted by Aoyama et al. (2018), non-glycosylated flavonoids exhibited tyrosinase inhibitory effects like kojic acid. Additionally, it was demonstrated that feijoa leaf extract has a strong capacity to inhibit key enzymes in diabetes, such as α-glucosidase and α-amylase. This notable antidiabetic activity could be attributed to the abundance of phenolic compounds, flavonoids, and triterpenoids in the feijoa leaf extract, which are recognized as antidiabetic agents (Saber et al., 2021).

3. Conclusions

In summary, this review highlights the complexity and diversity of secondary metabolites present in *Feijoa sellowiana*, as well as its various biological activities. The species reveals a rich variety of volatile and phenolic compounds in different parts, with unique chemical profile and distinct properties depending on several intrinsic and external factors. The choice of extraction methods and environmental conditions influence the chemical composition, emphasizing the importance of considering these factors in future studies and industrial applications. Furthermore, the antioxidant, antimicrobial, antiproliferative, and enzyme inhibitory properties suggest the potential of *Feijoa sellowiana* for a wide range of applications in food, pharmaceuticals, and cosmetics. Ultimately, this review provides a comprehensive insight into

the plant and its bioactive compounds, opening new avenues for research and development in this field.

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Chapter 2. Leaf Cuticular Waxes: chemical constituents in Brazilian genotypes of *Feijoa sellowiana* (synonym *Acca Sellowiana)* - identification and comparison

Abstract

Secondary metabolites have been used throughout history in products for the food, pharmaceutical, cosmetic, automotive, and agricultural industries, acting as active or auxiliary formulation ingredients. Cuticular waxes, one of the main functional components of the cuticle, are widely used in various industries, mainly due to their hydrophobic nature. *Feijoa sellowiana*, Myrtaceae, presents a diverse chemical profile that makes it very attractive for research on secondary metabolites. Several volatile and phenolic compounds have been reported for this species; however, the compounds present in the cuticular waxes have not been studied in any of the plant organs, presenting an interesting field for exploration. The wax extraction was performed by consecutive immersions in dichloromethane, and the compounds were analyzed through gas chromatograph coupled to a mass spectrometer (GC-MS). The main goal was to identify the compounds present in the cuticular waxes of the leaves of four Brazilian genotypes (Alcântara, Mattos, Nonante, and Helena) and the wild specimens, and to perform the respective comparison of the chemical profiles. In all genotypes, fatty acids and primary alcohols were the most abundant classes of wax components, while ursolic acid (triterpene) and 1-hexacosanol (primary alcohol) were the main compounds. However, Mattos and Alcântara genotypes were clearly distinguished from the other genotypes. Mattos presented a wide diversity of compounds and predominance of primary alcohols, while Alcântara exhibited a lower diversity with higher presence of

fatty acids and absence of alkanes. For these two genotypes we suggest that breeding process affected the leaf wax chemical profile.

1. Introduction

Wax is one of the main functional components of the cuticle, along with cutin. They are present on the surface of aerial plant organs such as fruits, leaves, stems, and flowers, acting as a hydrophobic barrier that provides protection against abiotic and biotic factors that can threaten the plant's survival (Riederer and Muller 2008; Lewandowska et al., 2020).

Waxes, through chemical signaling or increased lipophilicity on the leaf surface, act as a protective barrier against infection by pathogenic fungi (Jenks, et al., 1998). In addition, they interfere with plant-insect interaction by preventing adherence of these organisms to the plant surface, as well as oviposition and feeding (Bosco, 2019). Also, they play a key role in reducing water loss in plants, which benefits species that need to survive during periods of drought, protecting them from lethal desiccation as well as UV radiation (Ni et al., 2012; Sharma et al., 2018, Bosco, 2019).

The chemical profile of waxes is characterized by the presence of open-chain compounds such as fatty acids, alkanes, alcohols, esters, ketones, and cyclic substances such as triterpenes (Roma and Santos, 2022). However, this composition can vary depending on the species, organ, plant age, season, and collection location (Trivedi et al., 2019; Santos et al., 2023). Similarly, wax profiles can vary between breeding genotypes. Souza (2010) showed that although the qualitative profiles of the *n*-alkanes were similar, quantitative differences were observed in leaf cuticular waxes among four peanut genotypes

and two wild species. Likewise, Sarkar (2023), comparing breeding and wild specimens of barley (*Hordeum vulgare*), observed that despite the similar cuticle ultrastructure, distinct composition was detected.

Waxes exhibit unique properties that make them widely used materials in various industries, primarily due to their behavior as lipophilic matrices. In the cosmetics field, waxes are employed in the development of lipsticks, hair creams, and moisturizers, thanks to their emollient properties. Additionally, taking advantage of their hydrophobic nature, they are used for coating candies and medications and are considered as an alternative to plastic. Moreover, they are utilized in products such as paints and polishes for automobiles, floors, and furniture. These diverse applications demonstrate the versatility of waxes and their ability to meet the needs of different industrial sectors (Jenks et al., 2010; Charumanee et al., 2017).

The most economically important vegetable wax is obtained from the carnauba palm. This wax is primarily composed of aliphatic esters and cinnamic acid diesters. Its notable hardness, high melting point, and low solubility make it a widely used material in various industrial applications that take advantage of its unique benefits. So far, synthetic waxes have not been able to match its properties, which has led to ongoing research for alternatives (Zlokarnik et al., 2012).

The *Feijoa sellowiana,* also known as *Acca sellowiana*, is a species native to southern Brazil and belongs to the Myrtaceae. Although numerous volatile and phenolic compounds have been identified in this species, as far as we know, the composition of the cuticular wax of *Feijoa* has not been investigated. This

unexplored aspect offers a fascinating field of research, as the wax could potentially contain secondary metabolites with economic value, thus opening new opportunities for utilization. The fruit is the best known part of the species due to its nutritional and sensory properties, being the basis for the elaboration of a wide variety of food products, such as juices, jams and yogurts (Phan et al., 2019). In addition to its application in gastronomy, feijoa is used in the formulation of some cosmetic products (Zhu et al., 2018). Although commercial production of feijoa is mainly concentrated in New Zealand, Colombia, Azerbaijan, Georgia, and the United States (California), there is a growing interest in developing this industry in Uruguay and Brazil (dos Santos et al., 2009).

Research on Myrtaceae species such as *Corymbia citriodora, Eucalyptus gunnii*, and *Eucalyptus globulus* has revealed the predominant presence of β-diketones and sterols in leaf cuticular waxes (Rocha et al., 2024). Additionally, Koch (2006) identified triterpenoids such as beta-amyrin, betulin, ursolic acid, oleanolic acid, and the β-diketone triacontane-14,16-dione as the main components in *Eucalyptus gunnii* leaf wax. Li (1997) reported that long-chain β-diketones were predominant in most *Eucalyptus* species (subgenus *Symphyomyrtus*). However, in other species such as *E. barberi*, *E. ovata*, *E. johnstonii*, *E. subcrenulata*, and *E. vernicosa*, high proportions of long-chain hydrocarbons, aldehydes, or triterpenoids were observed. Finally, Wollenweber (2000) evaluated species from *Callistemon*, *Melaleuca*, and *Metrosideros*, finding a significant proportion of leaf waxes composed of triterpenoids, with a characteristic presence of flavonoids.

The objective of this study is to identify the compounds present in the leaf cuticular waxes of *Feijoa sellowiana* from Brazilian genotypes (Alcântara, Mattos, Nonante, and Helena), as well as wild specimens. Additionally, chemical profiles will be compared to establish whether the breeding process influenced the diversity and concentration of the wax compounds. Waxes were extracted by immersion in dichloromethane, and the compounds analyzed using gas chromatography-mass spectrometry (GC-MS).

2. Material and methods

2.1 Material

Leaves of *Feijoa sellowiana* of the genotypes Alcântara, Helena, Mattos, Nonante and wild specimens were obtained at the Active Germplasm Bank (BAG) from the São Joaquim Station of the Agricultural Research and Rural Extension of Santa Catarina - EPAGRI. In all cases, a sample consists of a mixture of health and fully expanded leaves of two adult specimens. The sample size is 25, composed of 5 repetitions of each genotype and 5 repetitions of the wild species.

2.2 Extraction of the cuticular waxes

Ten dried leaves of each sample were previously digitized to calculate the leaf area with the help of Image J 1.49v software.

The extraction process consisted of three consecutive dips, each one of 30 s, in dichloromethane (modified from Fernandes et al., 1964). The extracts were filtered and concentrated in rotary evaporator under reduced pressure until complete evaporation of the solvent. The wax was transferred to vials of known mass which were left to complete dry before weighted. The total wax content wax expressed in $\mu g.cm^{-2}$.

2.3 Derivatization process

Samples of approximately 1 mg of wax were derivatized using 50 µL of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 50 µL of pyridine, heated at 70°C for 1 hour (adapted from Jetter et al., 2000).

2.4 Analysis of the cuticular waxes

Samples were analyzed on a gas chromatograph coupled to mass spectrometer (GC-MS), using the Agilent 6850/Agilent 5975C equipment. An Agilent HP5-MS column (30 mx 250 µm film, 0.25 µm) was used for the analysis, with the initial temperature set at 100 °C for 5 minutes followed by heating at 5 °C/min until the final temperature of 320 °C, maintained for 8 minutes. The injection volume was 1 µL with helium as carrier gas at a constant flow rate of 1 mL/min. The temperatures of the injector, ion source, and quadrupole were 300 °C, 230 °C, and 150 °C, respectively. MS detection was performed with 70 eV electron ionization (EI) in full scan acquisition mode in a range of *m/z* 50 to 700 at 2.66 scans/s. The total analysis time was 57 minutes.

The wax compounds were identified by analyzing the fragmentation pattern of the resulting mass spectra, comparing them with those available in the literature and with the mass spectra available in the NIST 20.0 digital library.

2.5 Data pretreatment

Only compounds detected in at least 4 replicates were considered. An imputation process (mean) was performed for the missing data before the statistical analysis.

2.6 Statistical Analysis

All data are presented as mean values of n=5 (M) ± standard deviation (SD). The statistical significance of the obtained results was determined using the Kruskal-Wallis test, along with the Duncan test, if applicable. RStudio software was used for statistical analysis. Values of $p < 0.05$ were considered statistically significant.

A principal component analysis (PCA) was carried out using the relative percentage of all compounds identified in cuticular wax.

3. Results and discussion

3.1Total wax content

The Kruskal-Wallis test was performed to verify if there were statistically significant differences between the mean total wax content found in genotypes and wild species. The p obtained was 0.095, indicating insufficient evidence to reject the null hypothesis (Figure 1). The total amount of leaf cuticular waxes did not vary between genotypes and the wild plant, ranging from 52.9 to 70.1 $\mu g/cm^2$.

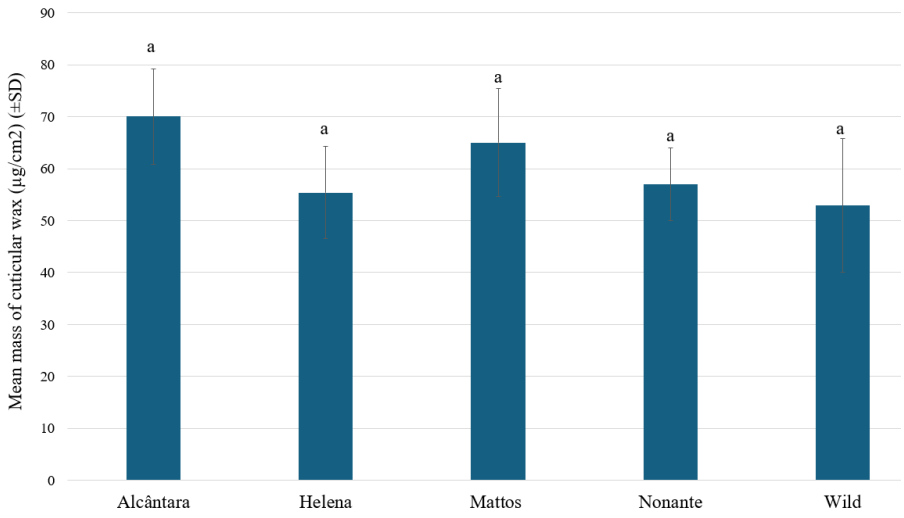

Figure 1. Mean of leaf cuticular waxes content of *Feijoa sellowiana* inbreeding genotypes Alcântara, Helena, Mattos and Nonante and wild plants. Data are shown as the means ± standard deviation (n = 5). There is not significant difference among the samples based on Kruskal-Wallis test ($p < 0.05$).

In *Eucalyptus grandis* genotypes (Myrtaceae), the average total wax content values ranged between 0.03 mg/cm^2 and 0.07 mg/cm^2. It was found that genotypes with higher wax levels showed resistance to eucalyptus rust disease, related to the growth of the fungus *Puccinia psidii*, while genotypes with lower percentages were more susceptible to the disease (Viana et al., 2010).

The results of this study are within the range reported for *Eucalyptus grandis*, a species belonging to the same family. In addition, although there were no statistically significant differences between the feijoa genotypes and the control group, the content was slight higher for the breeding groups, except Helena genotype. This increase may be associated with the tolerance of the genotypes to anthracnose and gray rot (Ducroquet et al., 2007), two of the main diseases affecting the species. The life cycle and expansion of the causal fungi

is favored by the presence of water either through splashing and/or humidity in the environment. Xiong (2023) found that *Sorghum bicolor* leaf waxes are the first line of defense against *Colletotrichum sublineola*, the fungus responsible for sorghum anthracnose, a disease that causes considerable crop losses annually. Ku (2020) showed that the presence of wax on lettuce (*Lactuca sativa*) leaves plays a crucial role in preventing the adhesion of *Salmonella*, which comes from contaminated irrigation water. This bacterium is responsible for causing Salmonellosis, an infection that affects the intestinal tract when contaminated lettuce is consumed.

3.2 Comparison of chemical profiles

The compounds found (28) in the cuticular wax of the feijoa genotypes and the wild specimens (used as control) were grouped into six main classes: alkanes, esters, sterols, triterpenes, primary alcohols, and fatty acids. Fatty acids were the predominant class in all groups, with the wild specimens having the highest value (44.37%), while the Mattos genotype had the lowest (37.70%). Primary alcohols were the second most abundant class of compounds; the Mattos genotype stood out for having the highest value (35.52%), while Nonante had the lowest (25.73%). Thus, these two classes correspond to more than 65% of the total wax content (Figure 2) in all genotypes, including wild specimens.

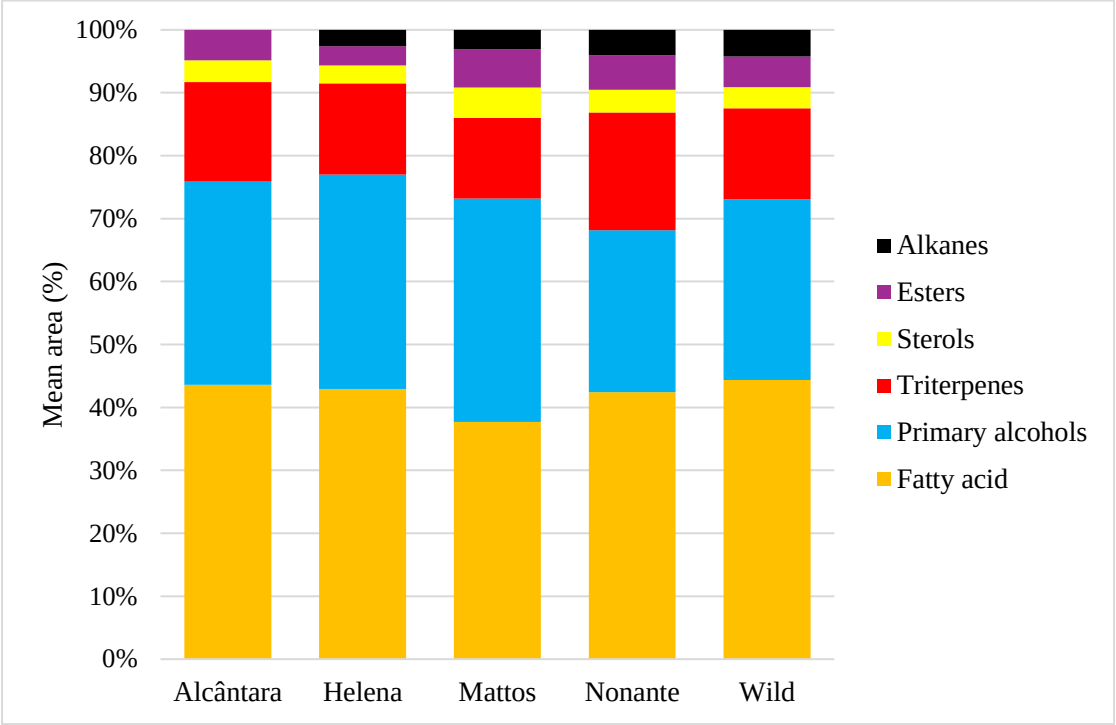

Figure 2. Main classes of leaf cuticular wax compounds of four inbreeding genotypes and wild specimens of *Feijoa sellowiana*.

Tripertenes were the third most abundant class, with the Nonante genotype having the highest percentage (18.7%), while Mattos had the lowest (12.8%). The percentage of other classes of compounds (esters, sterols, and alkanes) was less than 6% in each group. It is important to note that alkanes were not found in the Alcântara genotype. In general, we can state that the wax profile found in each case, although closely related to the conventional composition of cuticular waxes, differs from that reported for other Myrtaceae members, such as *Eucalyptus*, *Callistemon*, *Melaleuca* and *Metrosideros*, where β-diketones and triterpenes were the predominant classes (Wollenweber et al., 2000; Koch et al., 2006; Rocha et al., 2024).

Of the 28 identified compounds, 11 were fatty acids with homologues ranging from C16 to C32, 7 primary alcohols (C20 to C32), 4 alkanes (C25 to C31), 3 esters, 2 triterpenes and 1 sterol (Table 1). Mattos and Nonante were the most diverse genotypes with 27 compounds each, while only 18 were identified in Alcântara. In fatty acids, homologues with C24 and C26 chains were the most abundant, whereas in primary alcohols C26 predominated. Among the alkanes, the most abundant were those with C27 and C29 chains. These trends were constant in all groups.

The triterpene ursolic acid (No. 28, 12.89 - 19.30 %) and the primary alcohol, 1-hexacosanol (No. 22, 7.65 - 13.47 %) were the most abundant compounds in all groups, the former being the most abundant compound in Helena, Nonate and the control, while 1-hexanol was the most abundant in Alcântara and Mattos. Koch (2006) found that ursolic acid was one of the major compounds in the waxes of *Eucalyptus gunnii* leaves, while Viana (2010) identified the ursolic acid-derived triterpene, 3β-acetoxy-urs-12-en28-al, in *Eucalyptus grandis* genotypes.

Table 1: Concentration (% peak area) of the identified leaf cuticular wax compounds in four breeding genotypes and the wild specimens (control).

No.	Class/Compound	Molecular Formula	Genotypes*				Wild (control)
			Alcântara	Helena	Mattos	Nonante	
	Alkane						
1	Pentacosane	$C_{25}H_{52}$	nd	nd	0.65 ± 0.06	nd	nd
2	Heptacosane	$C_{27}H_{56}$	nd	1.37 ± 0.19^{a}	1.04 ± 0.14^{a}	1.07 ± 0.37^{a}	1.52 ± 0.25^{a}
3	Nonacosane	$C_{29}H_{60}$	nd	1.23 ± 0.15^{a}	1.39 ± 0.11^{a}	1.41 ± 0.55^{a}	1.44 ± 0.53^{a}
4	Hentriacontane	$C_{31}H_{64}$	nd	nd	nd	1.57 ± 0.63^{a}	1.25 ± 0.25^{a}
	Ester						
5	Heptacosyl acetate	$C_{29}H_{58}O_2$	nd	nd	1.86 ± 0.24^{a}	1.49 ± 0.27^{a}	1.65 ± 0.57^{a}
6	Heptacosyl benzoate	$C_{34}H_{60}O_2$	1.41 ± 0.31^{a}	1.02 ± 0.16^{a}	1.50 ± 0.20^{a}	1.21 ± 0.34^{a}	1.26 ± 0.37^{a}
7	Unidentified 1	-	3.44 ± 0.53^{a}	2.02 ± 0.71^{b}	2.74 ± 0.18^{ab}	2.74 ± 0.40^{ab}	2.01 ± 0.37^{b}
	Fatty acid						
8	Hexadecanoic acid	$C_{16}H_{32}O_2$	nd	1.31 ± 0.68^{a}	2.21 ± 0.88^{a}	2.68 ± 0.90^{a}	2.80 ± 0.72^{a}
9	Stearic acid	$C_{18}H_{36}O_2$	nd	nd	1.08 ± 0.27^{a}	1.64 ± 0.66^{a}	1.74 ± 0.34^{a}
10	Eicosanoic acid	$C_{20}H_{40}O_2$	1.41 ± 0.05^{ab}	1.74 ± 0.42^{ab}	1.15 ± 0.17^{b}	1.78 ± 0.13^{a}	1.85 ± 0.54^{a}
11	Docosanoic acid	$C_{22}H_{44}O_2$	3.64 ± 0.34^{a}	3.11 ± 0.59^{a}	2.19 ± 0.30^{a}	3.51 ± 0.37^{a}	3.55 ± 1.89^{a}
12	Tricosanoic acid	$C_{23}H_{46}O_2$	nd	1.19 ± 0.14^{a}	0.67 ± 0.12^{b}	1.00 ± 0.22^{ab}	1.23 ± 0.41^{a}
13	Lignoceric acid	$C_{24}H_{48}O_2$	13.25 ± 1.11^{a}	10.83 ± 1.96^{ab}	7.99 ± 0.87^{b}	10.73 ± 1.00^{ab}	10.61 ± 3.20^{ab}
14	Pentacosanoic acid	$C_{25}H_{50}O_2$	1.41 ± 0.40^{a}	1.75 ± 0.48^{a}	1.19 ± 0.25^{a}	1.35 ± 0.45^{a}	1.59 ± 0.40^{a}
15	Hexacosanoic acid	$C_{26}H_{52}O_2$	11.55 ± 1.09^{a}	8.95 ± 1.17^{b}	8.07 ± 0.47^{b}	7.62 ± 0.34^{b}	7.67 ± 0.82^{b}
16	Octacosanoic acid	$C_{28}H_{56}O_2$	8.01 ± 1.36^{a}	7.91 ± 1.24^{a}	7.98 ± 0.99^{a}	6.04 ± 1.54^{a}	7.32 ± 0.56^{a}
17	Triacontanoic acid	$C_{30}H_{60}O_2$	2.43 ± 0.73^{b}	3.75 ± 0.82^{a}	3.46 ± 0.51^{ab}	3.70 ± 0.71^{a}	3.97 ± 0.45^{a}
18	Dotriacontanoic acid	$C_{32}H_{64}O_2$	1.91 ± 0.54^{a}	2.42 ± 0.54^{a}	1.72 ± 0.19^{a}	2.40 ± 0.48^{a}	2.05 ± 0.44^{a}
	Primary alcohol						
19	1-Eicosanol	$C_{20}H_{42}O$	nd	0.81 ± 0.13^{a}	0.63 ± 0.09^{a}	0.79 ± 0.13^{a}	nd
20	Docosanol	$C_{22}H_{46}O$	nd	0.94 ± 0.13^{a}	0.82 ± 0.06^{a}	0.87 ± 0.12^{a}	nd
21	1-Tetracosanol	$C_{24}H_{50}O$	6.62 ± 1.04^{a}	5.17 ± 1.48^{a}	6.54 ± 0.18^{a}	3.97 ± 1.07^{a}	5.69 ± 2.44^{a}
22	1-Hexacosanol	$C_{26}H_{54}O$	13.47 ± 2.19^{a}	10.86 ± 2.16^{ab}	11.91 ± 0.72^{a}	7.65 ± 2.24^{b}	9.80 ± 2.91^{ab}
23	Octacosanol	$C_{28}H_{58}O$	7.66 ± 0.57^{a}	7.59 ± 0.50^{ab}	7.85 ± 0.56^{a}	5.03 ± 0.58^{c}	6.35 ± 1.10^{b}
24	Triacontanol	$C_{30}H_{62}O$	2.51 ± 0.10^{bc}	2.99 ± 0.27^{ab}	3.23 ± 0.32^{a}	2.24 ± 0.13^{c}	2.69 ± 0.39^{bc}
25	Dotriacontanol	$C_{32}H_{66}O$	2.01 ± 1.15^{a}	5.67 ± 1.91^{a}	4.54 ± 3.22^{a}	5.18 ± 2.86^{a}	4.20 ± 1.59^{a}
	Sterol						
26	Beta-Sitosterol	$C_{29}H_{50}O$	3.43 ± 0.84^{a}	2.85 ± 0.98^{a}	4.83 ± 1.17^{a}	3.63 ± 1.04^{a}	3.36 ± 1.59^{a}
	Triterpene						
27	Oleanolic acid	$C_{30}H_{48}O_3$	2.63 ± 0.40^{ab}	2.27 ± 0.56^{b}	2.54 ± 0.31^{ab}	3.47 ± 0.66^{a}	2.26 ± 0.57^{b}
28	Ursolic acid	$C_{32}H_{66}O$	13.23 ± 1.32^{ab}	12.24 ± 1.98^{ab}	10.23 ± 0.77^{b}	15.23 ± 1.96^{a}	12.15 ± 1.95^{ab}

* nd — not detected; Distinct letters in the same line indicate statistically significant difference ($p < 0.05$).

The Principal Component Analysis (PCA), as shown in figure 3, provides a detailed view of how the scores of the genotypes and the control group are distributed based on the relative abundance of the identified wax compounds (No. 1 - No. 28). PC1 explains 30.2% of the variability of the data, while PC2 explains 20.7%. The samples from genotypes Helena, Nonate, and the control group (wild) are not distinguishable based on wax components. Their phytochemical profiles were mostly similar with a greater diversity of compound classes. In contrast, Mattos and Alcântara genotypes are clearly separated from these groups, with Alcântara being the furthest away, characterized by a predominance of fatty acids, while Mattos shows a predominance of primary alcohols. Additionally, a greater proximity is observed among the replicates within these two groups. The distinction of Mattos and Alcântara genotypes may be closely related to the breeding process carried out, as suggested by Souza (2010) and Sarkar (2023) using peanut and barley, respectively.

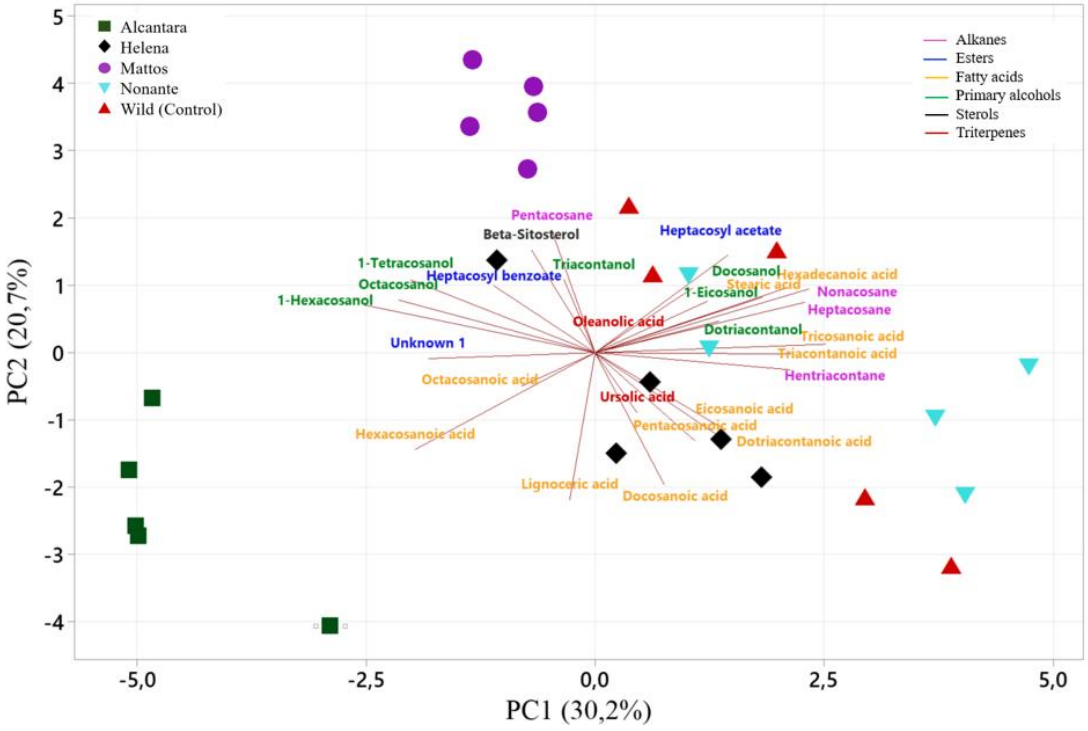

Figure 3. Principal component analysis (PCA) for the multivariate association between secondary metabolites and four varieties of *Feijoa sellowiana* and the control species.

4. Conclusions

We determined for the first time the chemical profile of the foliar waxes of *Feijoa sellowiana* for the Alcântara, Helena, Mattos and Nonante genotypes (developed by EPAGRI), together with the wild specimens, aiming verify whether the domestication process carried out affected the wax composition. In all cases, fatty acids and primary alcohols represented more than 65% of the total wax. The most abundant compounds were ursolic acid (triterpene) and 1-hexacosanol (primary alcohol) in all groups. The Mattos and Alcântara genotypes exhibited notable disparities in chemical composition. Mattos was distinguished by its wide diversity of compounds, with a predominance of

primary alcohols, while Alcântara showed a lower diversity, with a higher presence of fatty acids and absence of alkanes. Based on these results, at least for Mattos and Alcântara genotypes, these differences could be directly attributed to the breeding process carried out.

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Chapter 3. Chemical profile of the volatile oils of Brazilian genotypes of *Feijoa sellowiana* (synonym *Acca sellowiana*)

Abstract

Essential oils are known for their richness in aromatic compounds, which play fundamental roles in plant biology and have various industrial and medicinal applications. *Feijoa sellowiana* is a species native to southern Brazil, cultivated in different parts of the world. Numerous research studies have sought to determine the chemical profile of the species, revealing a rich diversity of compounds. In the case of essential oils, studies have mainly focused on the fruit, leaving a gap in knowledge about other parts of the plant such as the leaves. Therefore, this study aimed to identify the compounds present in the volatile oils of the leaves of four Brazilian genotypes (Alcântara, Helena, Mattos, and Nonante) developed by EPAGRI - Agricultural Research and Rural Extension Company of Santa Catarina, Brazil, comparing them with the profile of the wild specimens, to evaluate whether genetic improvement has influenced the composition of the oils. Distillation and gas chromatography-mass spectrometry techniques were used to extract and characterize the compounds. The results revealed qualitative similar general chemical profiles, with sesquiterpenes and monoterpenes as predominant classes. However, significant quantitative differences were observed among the genotypes. Both Alcântara and Helena genotypes exhibited the highest percentages of sesquiterpenes, reaching values of 90.6% and 90.4%, respectively, while Mattos genotype showed the highest percentage of monoterpenes (24.1%). The main sesquiterpenes identified were bicyclogermacrene, germacrene D and caryophyllene, with the notable inclusion of ocimene (monoterpene) as the main

compound in the Mattos genotype. Helena and Nonante genotypes along with the control group are not distinguished based on volatile oil. On the other hand, Mattos genotype was clearly distinguished by its wide diversity of compounds, with predominance of monoterpenes, while Alcântara is differentiated by a higher presence of oxygenated sesquiterpenes. Once all samples were collected in the same day and location, we suggest that these differences could be directly attributed to the breeding process.

1. Introduction

Volatile oils have a great diversity of aromatic compounds that provide specific odors and flavors. They can be found in flowers, leaves, fruits, seeds and roots, and their chemical composition can be very varied and complex. Phytochemical profiles of oils commonly contain terpenes, alcohols, ethers, aldehydes, ketones, esters, amines, amides, phenols, heterocycles, etc (Dhifi et al., 2016; Basavegowda et al., 2021). These compounds play a variety of roles, from conferring distinctive sensory characteristics to potential therapeutic applications.

The chemical composition and yield of the oil can vary considerably due to factors inherent to the plant, environmental conditions, sample collection method, extraction methods used, etc. (Figueiredo et al., 2008; DeGroot, et al., 2021). Oils can be obtained by various methods, including hydrodistillation, solvent-assisted extraction (SAFE) and headspace solid-phase microextraction (HS-SPME), the first being the most widely used.

Essential oils in plants play distinctive roles in attracting pollinating insects, contribute to the protection of plant tissues from parasites and animal

predators, help in communication between individuals and participate in the seed germination process. In addition, they find applications in various sectors such as the food industry and perfumery to traditional medicine, aromatherapy, and agronomy. They have also been shown to have biological activity as antioxidants, anti-inflammatories, antimicrobials, etc. (Dhifi et al., 2016; Srinivasan et al., 2016).

In the case of *Feijoa sellowiana* (synonym *Acca sellowiana*), a species native to southern Brazil and belonging to the Myrtaceae family, the volatile oils were predominantly characterized by ester and alcohol compounds in fruits, while terpenes prevailed in leaves. Esters such as methyl benzoate and ethyl benzoate have been pointed out as the main compounds responsible for conferring the characteristic aroma of feijoa (Shaw et al., 1983; Baena-Pedroza et al., 2020).

Terpenes, both monoterpenes and sesquiterpenes, have been consistently identified as the predominant class of compounds in investigations on the chemical composition of the volatile oils of feijoa leaves. For example, Mosbah (2018) identified limonene (36.2%), β-caryophyllene (27.8%), aromadendrene (12.5%), and α-copane (6.6%) as the major compounds. In addition, Cebi & Sagdic (2022) reported limonene and caryophyllene as the major compounds, with percentages of 33.46% and 22.18%, respectively, while El-Nashar (2022) identified caryophyllene oxide (24.3%), linalool (7.9%), and spaturenol (6.6%) as the main compounds present in feijoa volatile oils.

It is important to note that most of the available studies on the volatile oils of this species have been carried out with varieties cultivated in countries

such as Colombia, New Zealand, Turkey, Italy, and Egypt, focusing on the fruit. This has resulted in limited information on the chemical profiles of other parts of the plant, as well as a lack of data on the phytochemical profiles of genotypes and the wild specimens from Brazil.

The objective of this study is to identify the compounds present in the volatile oils of *Feijoa sellowiana* leaves from four specific Brazilian genotypes: Alcântara, Mattos, Nonante and Helena, developed by EPAGRI Agricultural Research and Rural Extension Company of Santa Catarina, Brazil, in addition to the wild specimens (control group). The aim is to compare the chemical profiles obtained, to evaluate whether the genetic improvement carried out in the genotypes had an impact on the composition of volatile oils. The extraction of the oils will be performed by hydrodistillation using a Clevenger apparatus, followed by analysis of the compounds by gas chromatography-mass spectrometry (GC-MS).

2. Material and methods

2.1. Material

Leaves of *Feijoa sellowiana* of four genotypes (Alcântara, Helena, Mattos, Nonante) and the wild specimens were obtained at Active Germplasm Bank (BAG) from the São Joaquim station of the Agricultural Research and Rural Extension of Santa Catarina - EPAGRI. In all cases, a sample consists of a mixture of leaves of two adult specimens. The sample size is 25, composed of 5 repetitions of each genotype and 5 repetitions of the wild specimens. The leaves were harvested and allocated in ice-bath in the field. In the laboratory, samples were stored in a -20 ºC freezer until the analysis.

2.2 Extraction of the volatile oils

Leaves (50 g) were crushed and transferred to a distillation flask connected to a Clevenger extractor. The plant material was covered with distilled water and heated for at least 4 hours. 300 µL of toluene was used to extract the oil due to the low amount. The sample was transferred to a 2 mL microtube and kept frozen until the analysis.

2.3 Analysis of the volatile oils

The oil samples were analyzed by Gas Chromatography coupled with Mass Spectrometry (GC/MS). An Agilent GC/MS system (6859/5975B) was used with a DB-5 HT capillary column (30 m x 0.25 mm x 0.25µm), employing helium as the carrier gas at a flow rate of 1 mL/min. The injector temperature was set at 250 °C, and the column heating ramp followed this program: 40 °C for 1 min, an increase of 6 °C/min up to 100 °C, and then an increase of 3 °C/min up to 200 °C. The source temperature was 230 °C, and the quadrupole temperature was 150 °C. Detection was carried out in positive mode, and mass spectra were obtained by scanning ions with the electron multiplier voltage adjusted to 70 eV, ranging from 50 to 800 *m/z*, at a scan rate of 2.66 scans per second (Savietto, 2011).

2.4 Identification of the volatile oils

The components were identified by comparing the mass spectra with those in the NYST library (version 20.0) and calculating the Linear Retention Index (LRI) as described by Viegas & Bassoli (2007). For this purpose, a standard mixture of *n*-alkanes (Sigma-Aldrich C8- C20) was used. The retention times of these alkanes served as an external reference standard to calculate the

LRI, together with the retention times of each compound of interest, according to the following equation:

$$LRI = 100 \times [((tc - tn)/(tn+1 - tn))+n]$$

Where: LRI - Linear Retention Index; tc - retention time of the compound of interest; tn - retention time of the hydrocarbon prior to the compound of interest; tn+1 - retention time of the hydrocarbon after the compound of interest; n - number of carbons in the prior hydrocarbon.

All LRI values obtained were compared with those from Adams (2009).

2.5 Data pretreatment

Only compounds detected in at least 4 replicates were considered. An imputation process (mean) was performed for the missing data before the statistical analysis.

2.6 Statistical Analysis

All data are presented as mean values (M) ± standard deviation (SD). The statistical significance of the obtained results was determined using the Kruskal-Wallis test, along with the Duncan test, if applicable. RStudio software was used for statistical analysis. Values of $p < 0.05$ were considered statistically significant.

A principal component analysis (PCA) was carried using the relative percentage of each compound.

3. Results and discussion

A total of 73 compounds distributed in seven main chemical classes were detected, of which 49 were sesquiterpenes (28 hydrocarbons and 21

oxygenated), 15 monoterpenes (9 hydrocarbons and 6 oxygenated), together with 3 ketones, 3 cycloalkanes, 1 aldehyde, 1 alkadiene, and 1 flavonoid. In the Mattos genotype 63 compounds were identified, in Nonante 57, in Alcântara 43, in Helena 41 and 44 in the wild specimens (control) (Figure 1).

Despite notable variability among compound classes, sesquiterpenes stood out as the predominant group, with a detection range of 31 to 41 compounds (hydrocarbons and oxygenated) in each genotype. The Mattos genotype exhibited the highest number of compounds. Additionally, this genotype stood out for having a greater number of monoterpenes (15) compared to the other genotypes.

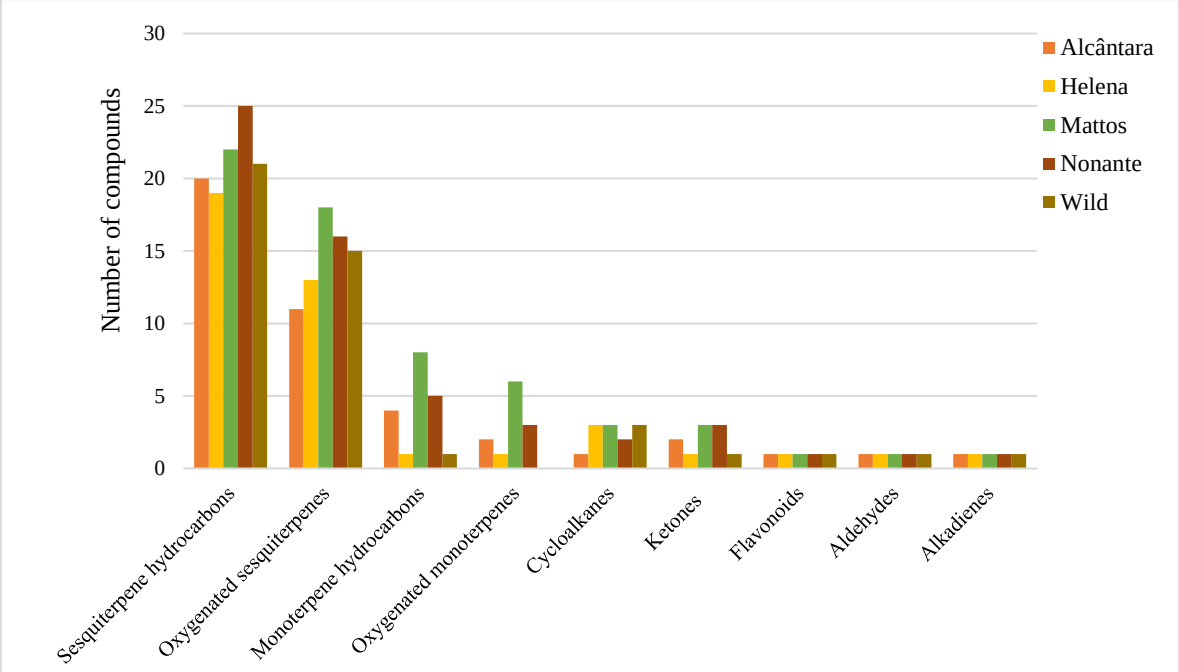

Figure 1. Distribution of compounds in essential oils of four genotypes of *Feijoa sellowiana* and the control specimens.

When analyzing the concentrations (relative abundance) of each class, it was also evident that both sesquiterpenes and monoterpenes were the most abundant, representing over 94% of the chemical profile of each genotype (Figure 2). The Alcântara and Helena genotypes showed the highest percentages of sesquiterpenes, with 90.6% and 90.5%, respectively, surpassing even the control, at 87%. On the other hand, whilst Mattos genotype exhibited the lowest percentage of this class of compounds at 71.0%, it presented the highest percentage of monoterpenes (24.1%), 2.5 times that of the control species (9.28%). It is important to note that, in all cases, the relative percentage of other classes of compounds was less than 2%.

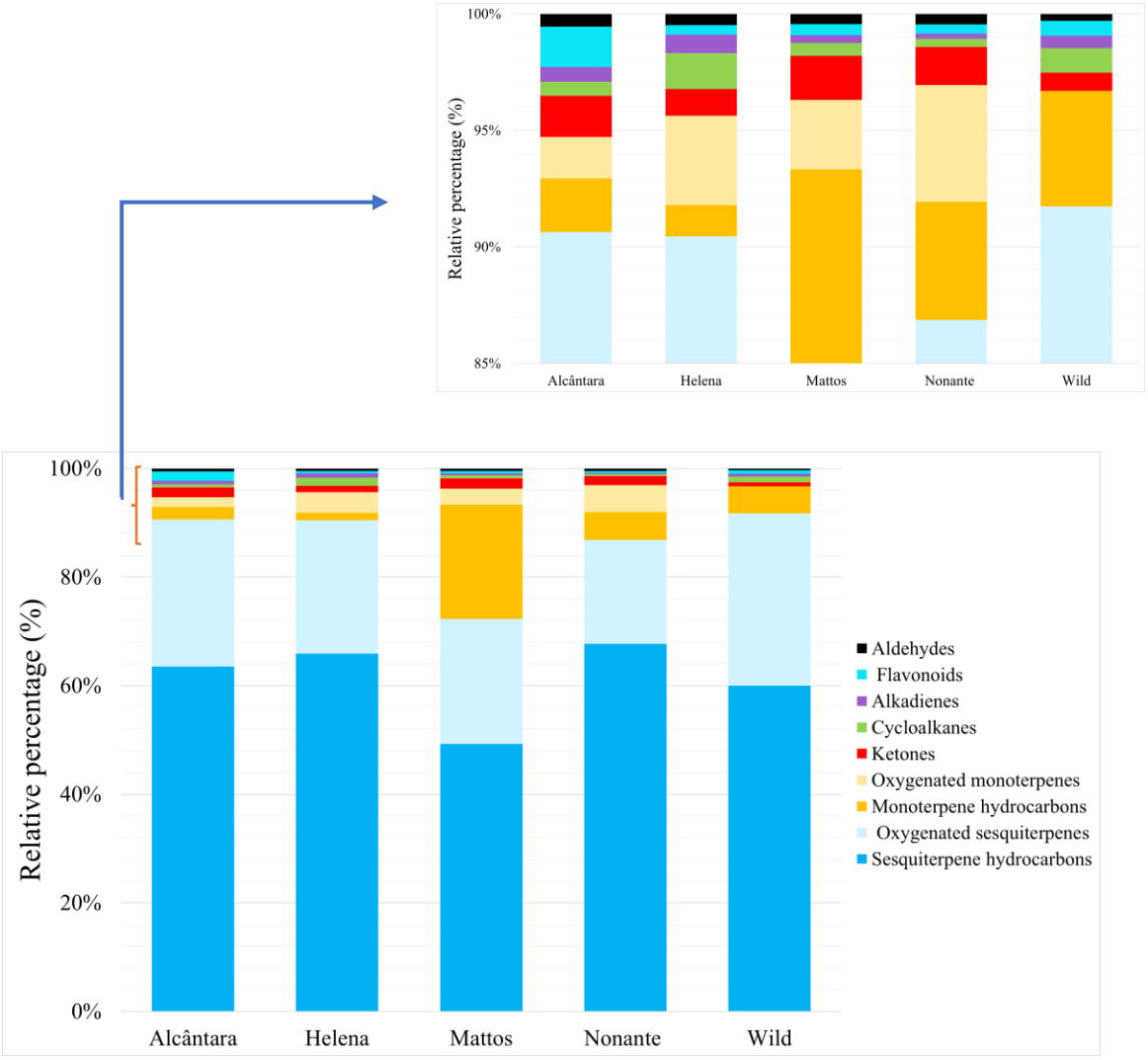

Figure 2. Chemical profile with the relative abundances of leaf essential oils classes of four genotypes and the wild specimens (control) of feijoa.

In research conducted with species of Myrtaceae, Dos Santos (2023) found that the leaf oils of *Eugenia* and *Psidium* are mainly composed of sesquiterpenes (>80%), while the oils of Syzygium *cumini* are abundant in monoterpenes (over 60%). Additionally, Nakamura (2010) revealed that oxygenated sesquiterpenes were the predominant class in the volatile leaf oil of several species such as *Eugenia acutata* (83.4%), *Eugenia candolleana*

(50.9%), *Eugenia opacabanensis* (54.3%), and *Myrcia splendens* (94.5%). In the specific case of feijoa, Elfarnini (2018) determined that 97.8% of the fruit peel oils were sesquiterpenes, while Motsbah (2018) observed that the leaf oil contained 57.8% sesquiterpenes and 39.9% monoterpenes. Therefore, the predominance of sesquiterpenes and monoterpenes in our study is in agreement with the available literature. Besides, non-oxygenated compounds were the most abundant, comprising 53-67% of the sesquiterpenes and 18-31% of the moterpenes identified (Figure 2).

The sesquiterpenes bicyclogermacrene (No. 1, 12.89 - 19.30 %), germacrene D (No. 2, 6.05 - 10.63 %), and beta-caryophyllene (No. 3, 8.08 - 13.87 %) were identified as the main compounds in Alcântara, Helena and Nonante genotypes, as well as in the control group (Figure 3; Annex 1). However, in the Mattos genotype, the monoterpene trans-ocimene (No. 50, 16.20 %) prevailed as the most abundant compound, followed by the previously mentioned sesquiterpenes. This finding suggests that the process of genetic improvement could have influenced the chemical profile the essential oils of Mattos genotype.

Despite the lack of previous research on leaf essential oils of Brazilian genotypes, it was observed that the sesquiterpene beta-caryophyllene was mentioned as a major compound in research conducted on varieties from Tunisia (Mosbah, 2018), Turkey (Cebi & Sagdic, 2022), as well as in Egypt (El-Nashar et al., 2022). It is relevant to note that the percentages obtained in those investigations significantly exceeded those found in this study.

The compounds germacrene D and ocimene were also found in the profiles of varieties from Tunisia and Turkey, albeit in relatively low concentrations (< 2%), which contrasts significantly with the results found for the Brazilian genotypes, where they are part of the major components. Bicyclogermacrene was not reported in any of the reviewed publications on essential oils from leaves. However, this sesquiterpene has been previously reported for the species as major components in the essential oils of the fruit, along with germacrene D (Binder et al., 1989; Fernández et al., 2004).

Among oxygenated sesquiterpenes, (-)-globulol (No. 29, 3.11 - 5.78 %) and spathulenol (No. 30, 3.47 - 4.85 %) were the most representative, while among monoterpenes, linalool (No. 59, 1.20 - 4.70 %) stood out. It is important to highlight that in Mattos genotype, the highest number of oxygenated monoterpenes was found. Linalool, spathulenol and caryophyllene oxide are some of the oxygenated compounds that have previously been identified as predominant in studies on feijoa leaves (Cebi & Sagdic, 2022; El-Nashar et al., 2022; Zhao et al., 2023).

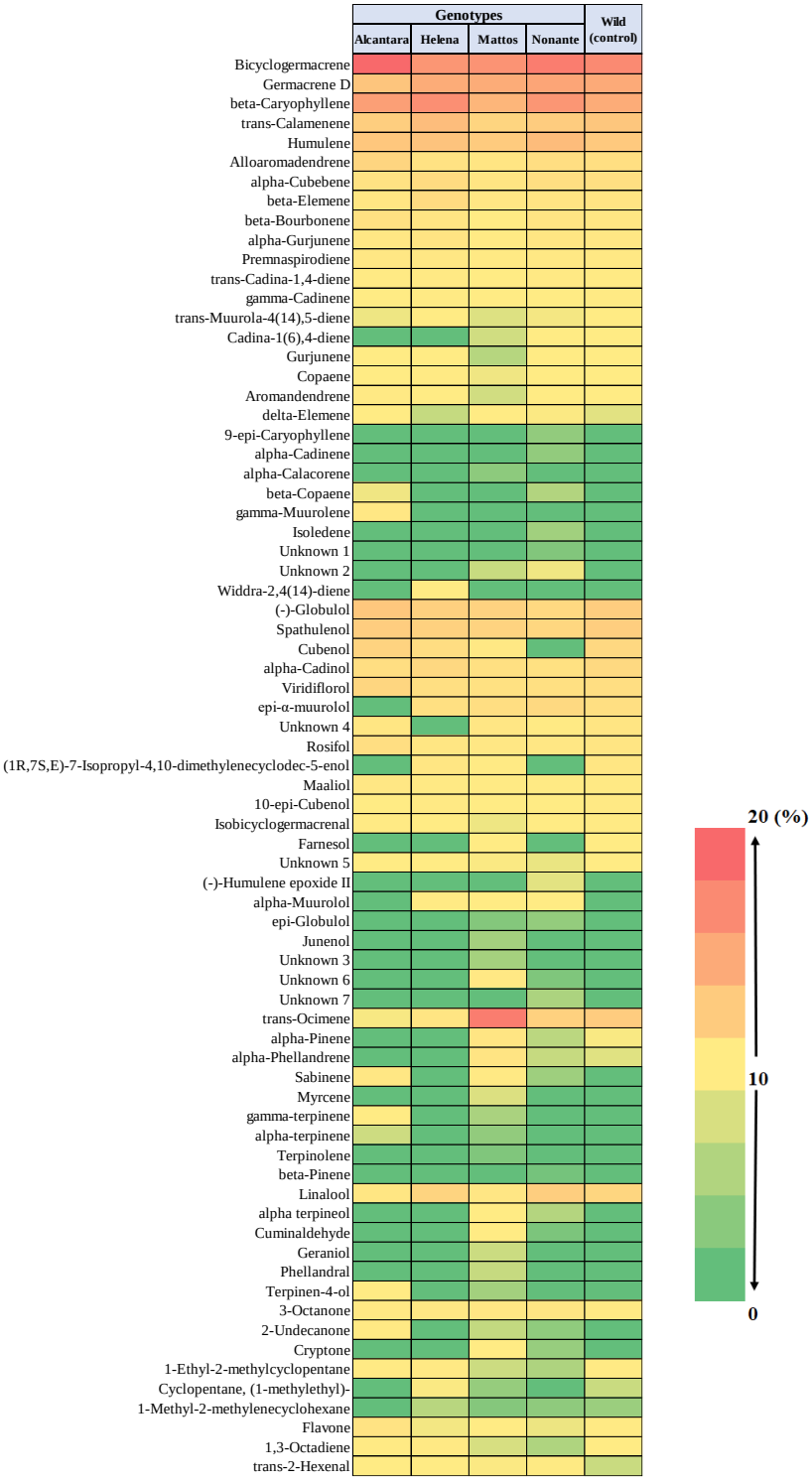

Figure 3. Heatmap with the relative abundances of each of the compounds identified in the feijoa essential oils from four *Feijoa sellowiana* genotypes and the control specimens.

A wide range of biological activities and industrial applications have been associated with the major compounds identified in feijoa volatile oils, such as bicyclogermacrene, germacrene D, beta-caryophyllene, and ocimene. For instance, bicyclogermacrene has been reported to exhibit antifungal, antimicrobial, allelopathic, cytotoxic, and acetylcholinesterase inhibitory activities (Santos et al., 2013; Durán-Peña et al., 2015), while germacrene D displays insecticidal activity against mosquitoes, repellent activity against aphids and ticks, and antimicrobial activity (Gil et al., 2016). Caryophyllene has been shown to have anticancer, analgesic, anticonvulsant, anti-inflammatory, gastroprotective, anxiolytic, antioxidant, and antimicrobial activities, and is used in the cosmetic and food industries as a flavoring agent (Hassan et al., 2020; Gupta et al., 2021), while ocimene has shown anticonvulsant, antitumor and antifungal activity (Russo et al., 2017). These results suggest the potential of feijoa volatile oils as a source of bioactive compounds with diverse applications.

Oxygenated mono and sesquiterpenes are also described with several biological activities, such as antitumor, anti-inflammatory, antibacterial, antiviral, antimalarial, antiallergic, neuroprotective, and antioxidant properties (Yang et al., 2020; Masyita et al., 2022). Furthermore, in plants, they perform vital functions, participating in attracting pollinators, protecting against herbivores, and acting as antibacterial agents (Song et al., 2023). Some oxygenated compounds, such as (-)-globulol or linalool, have been especially related to antimicrobial properties (Tan et al., 2008; Guo et al., 2021). On the other hand, compounds such as spathulenol have been linked to antioxidant,

anti-inflammatory and antitumor activities, especially in ovarian cancer (Passos et al., 2022).

The Principal Component Analysis (PCA) depicted in Figure 4 provides a detailed insight into the distribution of scores of genotypes and the control group concerning the concentrations of identified compounds of the leaf essential oils. PC1 explains 31.7% of the variability of the data, while PC2 explains 24.8%. Genotypes Helena, Nonante, and control group (wild specimens) display proximity to each other, indicating similarity in their essential oil profiles, primarily characterized by the predominant presence of sesquiterpenes hydrocarbons. In contrast, both Mattos and Alcântara are clearly separated from the others. Mattos genotype stands out for its high concentration of monoterpene compounds (hydrocarbons and oxygenated), suggesting a distinctive feature for this genotype, while Alcântara has the highest concentration of oxygenated sesquiterpenes.

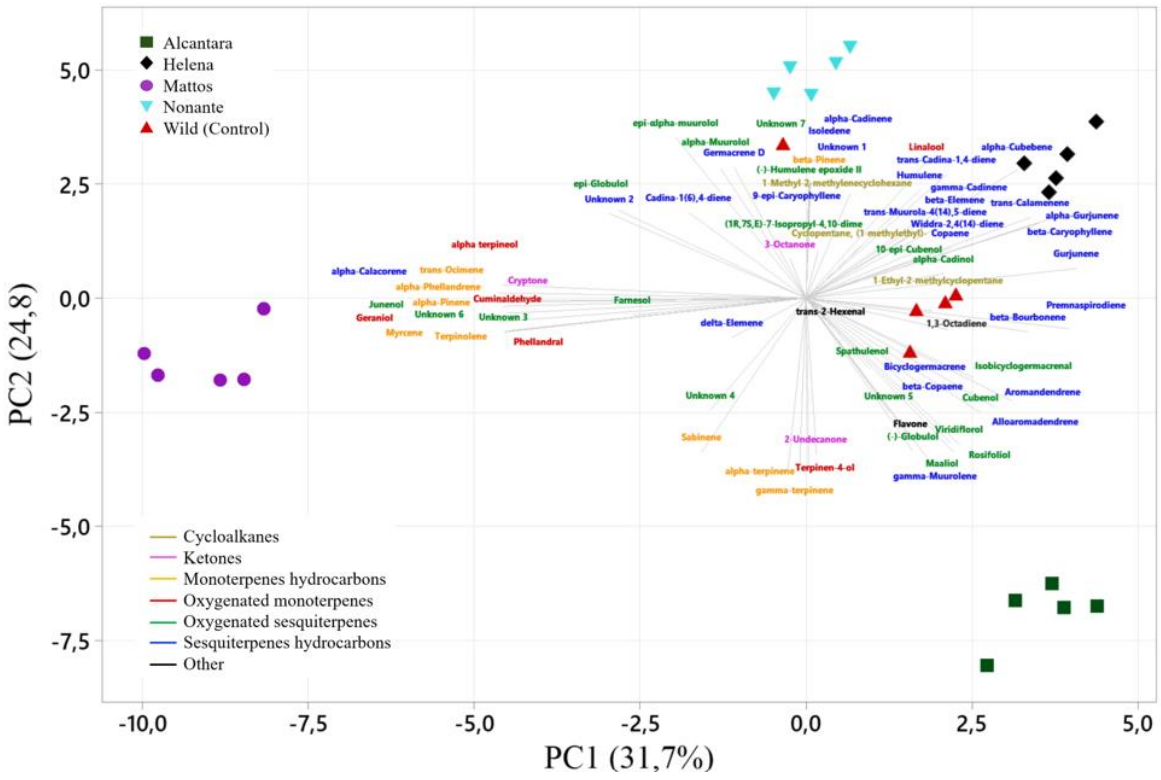

Figure 4. Principal component analysis (PCA) for the multivariate association between leaf essential oil components and four genotypes of *Feijoa sellowiana* and the control specimens.

In general terms, comparative studies between genotypes and wild species have indicated that discrepancies in the chemical composition of volatile oils are both qualitative and quantitative (El Bouzidi et al., 2013; Zhang et al., 2017). It has been observed that domesticated species tend to show better yields in volatile oil production (Abdellaoui et al., 2020). Factors such as genetics, geography, and agronomic practices have been identified as responsible for the variations found in the composition of these oils (Ghasemi et al., 2013; Abdellaoui et al., 2020). However, it is important to note that according to Dabbou (2011), differences may be attributed solely to genetic

factors if the conditions of collection, extraction process, and storage are uniform for all samples, as was done in the present study.

4. Conclusions

This study determined the chemical profile of volatile oils from the leaves of *Feijoa sellowiana* in Brazilian genotypes, Alcântara, Helena, Mattos, and Nonante (developed by EPAGRI), along with the wild specimens used as control. A wide variety of compounds were identified, classified into seven main chemical classes. Sesquiterpenes and monoterpenes emerged as the predominant classes in all cases. Additionally, the Mattos genotype exhibited trans-ocimene (a monoterpene) as the main compound, in contrast to bicyclogermacrene (a sesquiterpene) which predominated in the other groups. Helena, Nonante, and the control group had a predominant presence of hydrocarbon sesquiterpenes and are not distinguished based on volatile oil. Mattos and Alcântara genotypes showed notable disparities in chemical composition. While Mattos was distinguished by its wide diversity of compounds, with predominance of monoterpenes (hydrocarbons and oxygenated), Alcântara showed a higher presence of oxygenated sesquiterpenes. These differences could be directly attributed to the breeding process carried out on these genotypes, which could aid in the selection process.

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Supplementary material

Annex 1.

Table 1: Concentration (% peak area) of the identified compounds in four genotypes and the wild specimens (control).

LRI: Linear Retention Index; nd—not detected; a, b, c, d—indicators for statistical significance among genotypes ($p < 0.05$), identical letters indicate no statistically significant difference.

No.	Compound	LRI	Genotypes				Wild (control)
			Alcântara	Helena	Mattos	Nonante	
	Sesquiterpene hydrocarbons						
1	Bicyclogermacrene	1492	19.30 ± 3.08 ^{a}	12.89 ± 1.31^{bc}	13.21 ± 0.56 ^{bc}	16.43 ± 0.87 ^{c}	14.57 ± 3.65 ^{ab}
2	Germacrene D	1473	6.05 ± 1.34 ^{b}	9.71 ± 1.18 ^{ab}	9.60 ± 2.78 ^{ab}	10.63 ± 0.92 ^{a}	9.98 ± 3.82 ^{ab}
3	beta-Caryophyllene	1408	11.58 ± 0.64 ^{b}	13.87 ± 0.84 ^{a}	8.23 ± 0.52 ^{d}	12.83 ± 0.25 ^{ab}	9.63 ± 1.04 ^{c}
4	trans-Calamenene	1516	4.72 ± 0.45 ^{c}	7.17 ± 0.37 ^{a}	3.70 ± 0.33^{d}	4.96 ± 0.10 ^{c}	5.90 ± 0.75 ^{b}
5	Humulene	1443	5.73 ± 0.37 ^{a}	6.31 ± 0.33 ^{a}	5.12 ± 0.42 ^{a}	7.36 ± 0.28 ^{a}	5.49 ± 0.75 ^{a}
6	Alloaromadendrene	1449	3.66 ± 0.59 ^{a}	1.76 ± 0.19 ^{bc}	1.38 ± 0.15 ^{c}	2.38 ± 0.19 ^{b}	2.29 ± 0.67 ^{b}
7	alpha-Cubebene	1353	1.80 ± 0.23 ^{c}	2.78 ± 0.24 ^{a}	1.39 ± 0.06 ^{d}	2.42 ± 0.21 ^{ab}	2.25 ± 0.38 ^{b}
8	beta-Elemene	1403	1.23 ± 0.08 ^{cd}	2.80 ± 0.12 ^{a}	1.18 ± 0.04 ^{d}	1.58 ± 0.06 ^{b}	1.52 ± 0.36 ^{bc}
9	beta-Bourbonene	1394	1.97 ± 0.64 ^{a}	1.42 ± 0.17 ^{ab}	0.51 ± 0.07 ^{b}	1.47 ± 0.29 ^{a}	1.30 ± 0.83 ^{ab}
10	alpha-Gurjunene	1395	1.14 ± 0.11 ^{b}	1.52 ± 0.19 ^{a}	0.69 ± 0.04 ^{c}	1.27 ± 0.14 ^{ab}	1.10 ± 0.19 ^{b}
11	Premnaspirodiene	1497	1.16 ± 0.16 ^{a}	1.14 ± 0.06 ^{a}	0.75 ± 0.03 ^{b}	0.98 ± 0.06 ^{a}	0.99 ± 0.16 ^{a}
12	trans-Cadina-1,4-diene	1524	0.62 ± 0.11 ^{ab}	0.78 ± 0.12 ^{a}	0.51 ± 0.01 ^{b}	0.84 ± 0.12 ^{a}	0.69 ± 0.17 ^{ab}
13	gamma-Cadinene	1505	0.47 ± 0.07 ^{b}	0.66 ± 0.04 ^{a}	0.46 ± 0.07 ^{b}	0.53 ± 0.04 ^{b}	0.62 ± 0.01 ^{a}
14	trans-Muurola-4(14),5-diene	1482	0.40 ± 0.06 ^{ab}	0.54 ± 0.03 ^{a}	0.35 ± 0.04 ^{b}	0.42 ± 0.02 ^{ab}	0.55 ± 0.20 ^{a}
15	Cadina-1(6),4-diene	1463	nd	nd	0.31 ± 0.01 ^{b}	0.46 ± 0.05 ^{a}	0.55 ± 0.11^{a}

16	Gurjunene	-	0.53 ± 0.11 [a]	0.56 ± 0.02 [a]	0.24 ± 0.04 [b]	0.53 ± 0.07 [a]	0.50 ± 0.14 [a]
17	Copaene	1384	0.47 ± 0.08 [a]	0.61 ± 0.13 [a]	0.41 ± 0.18 [a]	0.47 ± 0.05 [a]	0.47 ± 0.04 [a]
18	Aromandendrene	1425	0.81 ± 0.08 [a]	0.47 ± 0.07 [b]	0.32 ± 0.03 [c]	0.54 ± 0.05 [b]	0.47 ± 0.12 [b]
19	delta-Elemene	1340	0.46 ± 0.09 [a]	0.28 ± 0.03 [a]	0.45 ± 0.25 [a]	0.44 ± 0.03 [a]	0.37 ± 0.07 [a]
20	9-epi-Caryophyllene	1454	nd	nd	nd	0.14 ± 0.01	nd
21	alpha-Cadinene	1528	nd	nd	nd	0.14 ± 0.01	nd
22	alpha-Calacorene	1534	nd	nd	0.12 ± 0.01	nd	nd
23	beta-Copaene	1429	0.41 ± 0.14 [a]	nd	nd	0.23 ± 0.03 [a]	nd
24	gamma-Muurolene	1465	1.04 ± 0.16	nd	nd	nd	nd
25	Isoledene	1378	nd	nd	nd	0.18 ± 0.02	nd
26	Unknown 1	-	nd	nd	nd	0.09 ± 0.01	nd
27	Unknown 2	-	nd	nd	0.29 ± 0.02 [b]	0.41 ± 0.05 [a]	nd
28	Widdra-2,4(14)-diene	1476	nd	0.66 ± 0.04	nd	nd	nd
	Oxygenated sesquiterpene						
29	(-)-Globulol	1579	5.78 ± 0.64 [a]	4.38 ± 0.56 [bc]	4.08 ± 0.53 [bc]	3.11 ± 0.08 [c]	4.89 ± 1.15 [ab]
30	Spathulenol	1574	4.85 ± 1.97 [a]	4.25 ± 1.30 [a]	3.98 ± 1.17 [a]	3.47 ± 0.79 [a]	4.74 ± 1.55 [a]
31	Cubenol	1637	3.96 ± 0.78 [a]	2.62 ± 0.61 [a]	1.03 ± 0.90 [b]	nd	3.23 ± 1.00 [a]
32	alpha-Cadinol	1654	2.36 ± 0.63 [a]	3.34 ± 0.36 [b]	2.18 ± 0.30 [b]	2.03 ± 0.26 [b]	3.15 ± 0.27 [a]
33	Viridiflorol	1587	3.63 ± 0.47 [a]	2.21 ± 0.21 [b]	1.99 ± 0.11 [b]	2.19 ± 0.08 [b]	2.45 ± 0.53 [b]
34	epi-alpha-muurolol	1640	nd	2.14 ± 0.18 [a]	2.47 ± 0.95 [a]	3.15 ± 0.23 [a]	2.33 ± 0.75 [a]
35	Unknown 4	-	1.22 ± 0.13 [ab]	nd	1.20 ± 0.32 [ab]	0.68 ± 0.21 [b]	1.45 ± 0.40 [a]
36	Rosifol	1597	2.34 ± 0.52 [a]	1.25 ± 0.12 [b]	1.05 ± 0.13 [b]	1.05 ± 0.04 [b]	1.44 ± 0.34 [b]
37	(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	1687	nd	1.21 ± 0.26 [a]	0.66 ± 0.02 [a]	nd	1.21 ± 0.63 [a]
38	Maaliol	1560	1.11 ± 0.20 [a]	0.75 ± 0.07 [b]	0.66 ± 0.05 [b]	0.65 ± 0.02 [b]	0.82 ± 0.18 [b]
39	10-epi-Cubenol	1625	0.56 ± 0.14 [ab]	0.74 ± 0.10 [a]	0.56 ± 0.02 [ab]	0.58 ± 0.08 [ab]	0.74 ± 0.11 [a]
40	Isobicyclogermacrenal	1736	0.67 ± 0.14 [a]	0.50 ± 0.09 [ab]	0.40 ± 0.01 [b]	0.47 ± 0.08 [ab]	0.63 ± 0.22 [ab]
41	Farnesol	1723	nd	nd	0.49 ± 0.04 a	nd	0.59 ± 0.13 a
42	Unknown 5	-	0.58 ± 0.16 [a]	0.45 ± 0.03 [ab]	0.44 ± 0.05 [ab]	0.39 ± 0.04 [b]	0.51 ± 0.11 [ab]

43	(-)-Humulene epoxide II	1603	nd	nd	nd	0.37 ± 0.06	nd
44	alpha-Muurolol	1645	nd	0.68 ± 0.08 [a]	0.50 ± 0.08 [b]	0.57 ± 0.03 [ab]	nd
45	epi-Globulol	1552	nd	nd	0.10 ± 0.01 [b]	0.14 ± 0.01 [a]	nd
46	Junenol	1614	nd	nd	0.19 ± 0.01	nd	nd
47	Unknown 3	-	nd	nd	0.19 ± 0.01	nd	nd
48	Unknown 6	-	nd	nd	0.91 ± 0.03 a	0.08 ± 0.03 b	nd
49	Unknown 7	-	nd	nd	nd	0.21 ± 0.02	nd
	Monoterpene hydrocarbons						
50	trans-Ocimene	1032	0.43 ± 0.15 [d]	1.34 ± 0.57 [cd]	16.50 ± 1.23 [a]	4.31 ± 0.95 [bc]	4.95 ± 3.45 [b]
51	alpha-Pinene	932	nd	nd	1.46 ± 0.36 [a]	0.26 ± 0.13 [b]	0.43 ± 0.24 [b]
52	alpha-Phellandrene	1002	nd	nd	1.48 ± 0.32 [a]	0.29 ± 0.09 [b]	0.36 ± 0.23 [b]
53	sabinene	972	1.06 ± 0.39 [a]	nd	0.81 ± 0.16 [a]	0.17 ± 0.05 [b]	nd
54	Myrcene	991	nd	nd	0.35 ± 0.06	nd	nd
55	gamma-terpinene	1060	0.51 ± 0.16 [a]	nd	0.20 ± 0.02 [b]	nd	nd
56	alpha-terpinene	1016	0.31 ± 0.11 [a]	nd	0.14 ± 0.02 [b]	nd	nd
57	Terpinolene	1089	nd	nd	0.09 ± 0.02	nd	nd
58	beta-Pinene	979	nd	nd	nd	0.05 ± 0.01	nd
	Oxygenated monoterpenes						
59	Linalool	1101	1.32 ± 0.40 [b]	3.84 ± 1.10 [a]	1.20 ± 0.39 [b]	4.70 ± 0.79 [a]	3.53 ± 1.34 [a]
60	alpha-terpineol	1185	nd	nd	0.56 ± 0.08 [a]	0.23 ± 0.06 [b]	nd
61	Cuminaldehyde	1235	nd	nd	0.45 ± 0.11 [a]	0.07 ± 0.02 [b]	nd
62	Geraniol	1249	nd	nd	0.30 ± 0.01	nd	nd
63	Phellandral	1272	nd	nd	0.29 ± 0.02	nd	nd
64	Terpinen-4-ol	1172	0.47 ± 0.12 [a]	nd	0.19 ± 0.01 [b]	nd	nd
	Ketones						
65	3-Octanone	987	1.02 ± 0.67 [a]	1.15 ± 0.38 [a]	1.06 ± 0.11 [a]	1.35 ± 0.32 [a]	0.77 ± 0.32 [a]
66	2-Undecanone	1291	0.75 ± 0.24 [a]	nd	0.28 ± 0.01 [b]	0.13 ± 0.04 [b]	nd
67	Cryptone	1181	nd	nd	0.55 ± 0.05 [a]	0.15 ± 0.05 [b]	nd
	Cycloalkanes						

68	1-Ethyl-2-methylcyclopentane	-	0.60 ± 0.33 [a]	0.85 ± 0.66 [a]	0.31 ± 0.23 [a]	0.22 ± 0.03 [a]	0.60 ± 0.49 [a]
69	Cyclopentane, (1-methylethyl)-	-	nd	0.44 ± 0.27 [a]	0.15 ± 0.15 [a]	nd	0.30 ± 0.21 [a]
70	1-Methyl-2-methylenecyclohexane	-	nd	0.25 ± 0.15 [a]	0.10 ± 0.07 a	0.13 ± 0.04 [a]	0.16 ± 0.11 [a]
	Flavonoids						
71	Flavone	-	1.72 ± 0.82 [a]	0.42 ± 0.08 [b]	0.47 ± 0.03 [b]	0.40 ± 0.18 [b]	0.64 ± 0.22 [b]
	Alkadienes						
72	1,3-Octadiene	-	0.64 ± 0.29 [ab]	0.79 ± 0.40 [a]	0.34 ± 0.26 [ab]	0.22 ± 0.04 [b]	0.53 ± 0.31 [ab]
	Aldehydes						
73	trans-2-Hexenal	850	0.55 ± 0.26 [a]	0.48 ± 0.18 [a]	0.44 ± 0.14 [a]	0.45 ± 0.48 [a]	0.29 ± 0.11 [a]

Chapter 4. Preliminary evaluation of leaf phenolic compounds and antioxidant activity of Brazilian genotypes of *Feijoa sellowiana* (synonym *Acca sellowiana*)

Abstract

Phenolic compounds are an important source of research due to their multiple health benefits. Known mainly for their powerful antioxidant properties, they act as agents capable of neutralizing free radicals and protecting cells from oxidative damage, thus preventing the onset of various types of diseases. In plants, they mainly serve as a defense against pathogens and herbivores. The chemical profile of phenolic compounds in *Feijoa sellowiana* leaves of Brazilian genotypes Alcântara, Mattos, Nonante and Helena, developed by EPAGRI, together with wild specimens, was investigated. The content of total phenols and the antioxidant capacity of the extracts obtained by ultrasonic-assited method with 80% methanol were evaluated. The compounds were analyzed by HPLC-DAD, the total phenolic compounds content was determined with the Folin-Ciocalteu reagent, and the antioxidant activity was evaluated with the DPPH reduction assay in crude extracts and partition phases. The content of total phenols in the leaves ranged from 10.33 ± 4.09 to 13.70 ± 5.35 mg GAE per gram of fresh extract, without differences between genotypes and wild species. Mainly derivatives of quercetin were identified in the crude extracts, with rutin and quercetin standing out for their potent antioxidant action. These findings may highlight the potential use of *Feijoa sellowiana* leaves in therapeutic and nutraceutical applications.

1. Introduction

Research on phenolic compounds has been of great interest due to their diversity and their health benefits as antioxidant agents. In the various physiological processes of the organism, numerous oxidative reactions take place and generate free radicals, such as superoxide anion (O_2.-), hydroxyl radical (OH), as well as non-radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Beyhan et al., 2010). These free radicals can cause significant deterioration in essential molecules such as nucleic acids, lipids, proteins, and carbohydrates, compromising their integrity and cellular function if they are not adequately removed from the body.

The resulting cell damage can trigger a wide range of diseases and accelerate the aging process. In response to this challenge, phenolic compounds emerge as crucial players by playing multiple roles as reducing agents, hydrogen donors, singlet oxygen scavengers, and metal chelators, which confer antioxidant properties (Liang et al., 2010). This outstanding capacity allows them to mitigate diseases by restoring physiological oxidative balance, influencing key biological pathways, and preserving the functional integrity of cell membranes.

Phenolic compounds are secondary metabolites that are widely distributed in the plant kingdom and are metabolized through the shikimate pathway and the malonate pathway (Marchiosi et al., 2020). In the context of plants, these compounds play key roles, including protection against predators, as they act as a first line of defense, as well as having roles as phytoalexins and allelopathic substances. In addition, they serve as attractants and repellents for various organisms, both for the flowering and pollination process and to avoid

invading pathogens (Azcón-Bieto et al., 2008; Pratyusha, et al., 2022). Besides these functions, phenolic compounds participate in processes associated with plant growth and development (Wallis et al., 2020).

The chemical profile of phenolic compounds in *Feijoa* (*Acca*) *sellowiana*, belonging to the Myrtaceae family, exhibits significant variations depending on the studied plant part. According to Karsli (2021), leaf extracts show a higher total content of phenolic compounds compared to other parts such as the peel and pulp of the fruit. Additionally, flowers and fruits, especially the peel, stand out for their rich content of anthocyanins, flavonols, and ellagitannins, while leaves display a diversity of phenolic acids and other flavonoids. Regarding antioxidant activity, available research on the species has identified a relationship between this activity and polyphenols, vitamin C, and flavonoids such as quercetin (Vuotto et al., 2000; Ebrahimzadeh et al., 2008; Zhu, 2018; Sánchez-Riaño et al., 2020).

The objective of this study was to investigate the chemical profile of phenolic compounds present in the leaves of four specific genotypes of *Feijoa sellowiana* from Brazil (Alcântara, Mattos, Nonante and Helena) developed by the Rural Research and Extension Company EPAGRI of Santa Catarina, Brazil, as well as in the wild species (control group). In addition, it is intended to evaluate the content of total phenols, as well as the antioxidant capacity of the extracts obtained. For extraction, dried leaves were pulverized and subjected to an exhaustive process of cold maceration with 80% methanol, followed by analysis of the compounds by high performance liquid chromatography with diode array detection (HPLC-DAD). The total content of phenolic compounds was determined by a colorimetric method using the Folin-Ciocalteu reagent,

while the antioxidant activity was evaluated with the DPPH reduction assay in the crude extract and in partition phases.

2. Material and methods

2.1. Material

Leaves of *Feijoa sellowiana* of the genotypes Alcântara, Helena, Mattos, Nonante and wild specimens were obtained at the Active Germplasm Bank (BAG) from the São Joaquim Station of the Agricultural Research and Rural Extension of Santa Catarina - EPAGRI. In all cases, a sample consists of a mixture of health and fully expanded leaves of two adult specimens. The total sample size was 25, composed of 5 repetitions of each genotype and 5 repetitions of the wild species.

2.2 Preparation of the crude extracts and partition phases

Dried leaves (1g) were pulverized and subjected to ultrasonic extraction with 5 mL of 80% methanol (3 times of 30 min). The extracts were gathered, filtered, and concentrated using a rotary evaporator giving rise to the crude extracts (CE).

To proceed the partition, 5 g of dried leaves of each replica of each genotype were pulled together (total = 25 g) and submitted to the same extraction procedure described above. This second CE obtained was dissolved in 20% methanol and partitioned with solvents of increasing polarity in the following order: hexane (Hx), dichloromethane (DCM), ethyl acetate (AcOEt), and butanol (BuOH). For each phase, three consecutive washes were performed with 150 mL of the corresponding solvent.

After this procedure, six distinct samples were available for each genotype: the crude extract (CE), the Hx, DCM, AcOEt and BuOH partition phases, and the residual hydroalcoholic (HA) phase.

2.3 High-performance liquid chromatography with diode array detector (HPLC-DAD)

The crude extracts (CE) and AcOEt and BuOH partition phases were dissolved in methanol HPLC-grade at 2 mg/mL. HPLC-DAD analyses were performed using the Agilent 1260 system. Chromatograms were obtained with detection at 352 nm, employing the Zorbax C18 column (150 mm x 4.6 mm x 3.5 μm) at 45°C. The mobile phase was composed of 0.1% acetic acid (AcOH) and acetonitrile (CH_3CN) and employed with the gradient elution begining with 10% CH_3CN (0-6 min), increasing to 15% (6-7 min), and remaining isocratic for 15 minutes, followed by an increase to 50% (22-32 min) and up to 100% (32-42 min), remaining isocratic for an additional 8 minutes. The solvent flow rate was kept constant at 1.0 mL/min, and 3 μL of sample was injected for analysis.

Compound identification proposals were based on the analysis of UV-visible absorption spectra and comparison of retention times and UV-visible spectra of standards.

2.4 Quantification of Total Phenolic Content

The total content of phenolic compounds was determined with a colorimetric method using the Folin-Ciocalteu reagent (Sigma-Aldrich), following the protocol described by Pires and collaborators (2017). Briefly, the samples (CE) were solubilized in methanol at a concentration of 20 mg/mL. Using a 96-well microplate, 200 μL of ultrapure water, 20 μL of Folin-Ciocalteu

reagent, 20 µL of each sample and 60 µL of saturated sodium carbonate solution were added in each well. After 30 min of incubation, the absorbances were recorded at 760 nm using a microplate reader (Synergy™ H1). Gallic acid was used as a reference standard for the calibration curve and the results were expressed as gallic acid equivalents/mg of extract. Each sample were added in triplicate in the microplate.

2.5 Antioxidant assay

The protocol was carried out in a 96-well microplate, and absorbance readings were taken using a microplate reader (Synergy™ H1).

An aliquot of 20 µL of CE and partition phases (1 mg/mL) was added to the wells containing 280 µL of DPPH reagent. After incubating the microplate for 20 minutes at room temperature, absorbance was measured at 515 nm. Trolox® was used as a reference standard for the calibration curve and the values were calculated as mg of Trolox per gram of extract (Pires et al. 2017a). Each sample were added in triplicate in the microplate.

2.6 Statistical Analysis

All data are presented as mean values of n=5 (M) ± standard deviation (SD). The statistical significance of the obtained results was determined using the Kruskal-Wallis test, along with the Duncan test, if applicable. RStudio software was used for statistical analysis. Values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

3.1 Total phenolic content (TPC)

The Kruskal-Wallis test was performed to determine if there were statistically significant differences in the average of TPC between genotypes and wild specimens of *Feijoa sellowiana*. The p value obtained was 0.7802, indicating insufficient evidence to reject the null hypothesis (Figure 1), suggesting that there were no statistically significant differences in TPC between genotypes and wild plants.

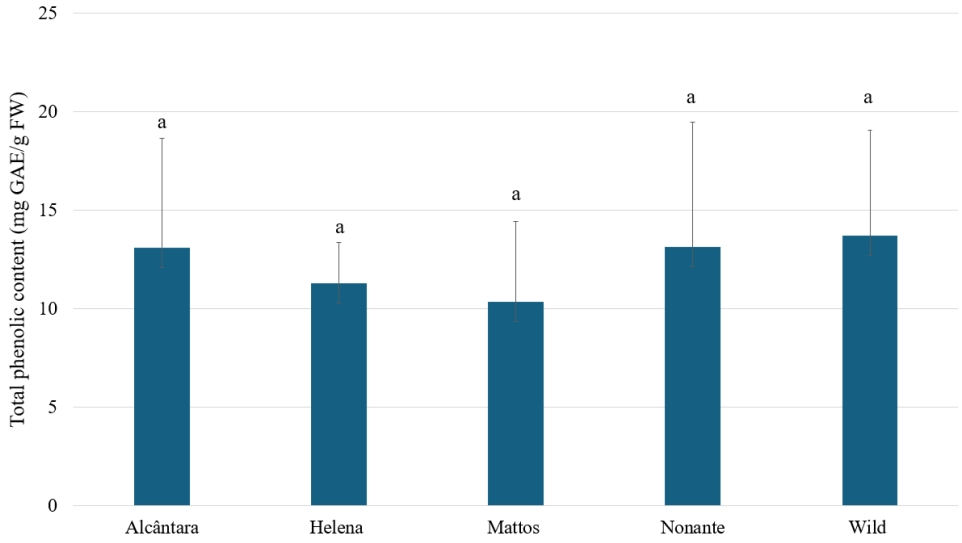

Figure 1. Mean of total phenolic content (TPC) of *Feijoa sellowiana* leaf extracts of inbreeding genotypes Alcântara, Helena, Mattos and Nonante and wild plants. Data are shown as the means ± standard deviation (n = 5). There is not significant difference among the samples based on Kruskal-Wallis test ($p < 0.05$).

The TPC in the leaves ranged from 10.33 ± 4.09 to 13.70 ± 5.35 mg of GAE per gram of extract. These results may indicate that, despite the breeding practices applied to the genotypes, the total phenolic content remained stable.

Available studies on *Feijoa sellowiana* leaf extracts have shown different results (Table 3 – Chapter I).

Mosbah (2019) and Karslı (2021) reported results closer to ours, with 9.48 ± 0.02 mg GAE/g of extract in plants from Tunisia and a range of 4.60 to 5.54 mg GAE/g extract using samples from Turkey, respectively. The late study also applied ultrasound-assisted extraction. In contrast, Beyhan (2010) and Mosbah (2018), studying samples from Turkey extracted by maceration, reported a significantly higher content of TPC, 68.69 mg GAE/g of extract or 179.43 ± 1.59 mg GAE/g extract, respectively.

Discrepancies in TPC can be attributed to biological factors, edaphic and environmental conditions, as well as to the extraction method and solvent used (Ksouri, et al., 2008; Tan et al., 2013). Nantitanon (2010) reported that *Psidium guajava* leaf extracts had a higher total phenolic content and antioxidant activity when the ultrasonication technique was used, while maceration without agitation produced the extract with the lowest total phenolic content. Irfan (2022) also reported higher values of polyphenol extraction yield (26.68%), TPC (61 mg GAE/g extract) and DPPH activity (73.85%) in *Cymbopogon citratus* leaf extracts obtained by ultrasonic-assisted method.

Notwithstanding, maceration seems to be more effective for phenolic extraction of feijoa leaves. TCP values of the present study and that from Mosbah (2019) and Karslı (2021) are lower than that from Beyhan (2010) and Mosbah (2018). It is worth to mention that both Mosbah (2018) and Karslı (2021) evaluated plants from Turkey.

A study by Amarante (2017) analyzed TPC of fruit peels and pulp of the same Brazilian genotypes of feijoa investigated in our study (Alcântara, Helena, Mattos and Nonante). Aqueous extracts presented higher TPC than hydroalcoholic extracts for both, peels, and pulp. Comparing plant parts, the fruit peels presented more phenolic compounds than fruit pulps. Helena and Mattos genotypes presented the lowest peel and pulp TPC values, respectively, while Nonante presented the highest value for both parts. Despite the absence of statistical difference of TPC in the present study, Helena and Mattos also presented the lowest values in leaves, suggesting that the breeding process of these two genotypes may have influenced the accumulation of total phenolics.

3.2 Comparison of chemical profiles and antioxidant activity

Distinct flavonoids were detected in the CE of *Feijoa sellowiana*. The suggested identification was based on retention times and comparison with standard compounds, besides UV-Vis absorption values (Table 1). The proposed compounds mostly correspond to quercetin derivatives, with a structure of glycosylated flavonols.

Table 1. Phenolic compounds detected (average relative percentage of peaks) in the crude extract of four inbreeding genotypes and wild specimens of *Feijoa sellowiana.*

#	Retention time (min)	λmax (nm)	Tentative identification	Genotypes				Wild (control)
				Alcântara	Helena	Mattos	Nonante	
1	1.517	224	Unknown 1	3.07 ± 0.66^{a}	2.99 ± 0.23^{a}	1.72 ± 0.55^{b}	2.89 ± 0.84^{a}	2.38 ± 0.41^{ab}
2	10,899	252, 356	Unknown 2	3.83 ± 0.74^{ab}	4.21 ± 0.78^{ab}	3.22 ± 1.23^{b}	6.20 ± 1.73^{a}	4.70 ± 1.58^{ab}
3	13,849	256. 354	Quercetin-3-O-rutinosídeo (Rutin)	34.69 ± 3.16^{a}	32.45 ± 5.15^{a}	31.96 ± 5.28^{a}	30.06 ± 5.91^{a}	29.50 ± 4.53^{a}
4	16,251	256, 354	Quercetin-3-O-xilosídeo (Reynoutrin)	7.08 ± 1.33^{a}	**	5.19 ± 1.27^{a}	5.92 ± 0.87^{a}	5.11 ± 0.61^{a}
5	17,224	256, 354	Quercetin-3-O-arabinosideo (Guajaverin)	22.74 ± 2.05^{a}	18.64 ± 2.19^{ab}	17.51 ± 1.87^{b}	20.17 ± 2.94^{ab}	18.82 ± 1.89^{ab}
6	18,562	256, 352	Quercetin derivative	28.58 ± 1.33^{a}	30.49 ± 3.42^{a}	25.38 ± 2.21^{a}	25.56 ± 3.01^{a}	30.10 ± 3.66^{a}
7	20,599	256, 248	Quercetin-3-O-rhamnosídeo (Quercitrin)	**	11.22 ± 2.27^{a}	10.87 ± 3.28^{a}	9.38 ± 2.87^{a}	9.40 ± 2.25^{a}
8	28,946	255, 370	Quercetin	**	**	4.15 ± 1.84	**	**

** Show the absence of peak. Distinct letters in the same line indicate significant differences among genotypes ($p < 0.05$).

Mattos was the most diverse genotype while Alcântara and Helena were the least diverse. Rutin, guajaverin and a one quercetin derivative (N. 6 – RT = 18,562 min) were the major compounds found in the crude extracts of the Brazilian genotypes and in the wild specimens (control).

Previous studies have reported a wide variety of phenolic compounds in this species. In leaves, quercetin and rutin have been mentioned in several investigations (Mousavi et al., 2018; Poodi et al., 2018; Bimakr et al. 2019; Saber et al., 2021; Cebi & Sagdic, 2022). Aoyama (2018), on the other hand, reported other quercetin derivatives, such as isoquercetin, guaijaverin, reynoutrin and quercitrin, but hyperoside was the most abundant flavonoid. Flavonol derivatives are also common in other species of Myrtaceae, such as in the genus *Myrcia* (Cascaes et al., 2015) and in *Psidium guajava* (Furlan et al., 2010; Sohafy, 2010).

The crude extracts and partition phases were subjected to antioxidant evaluation using the DPPH assay. In all cases the most polar partition phases (ethyl acetate and butanol) presented the best results (Table 2). Values ranged from 266.86 ± 1.67 to 270.71 ± 1.02 mg TE per gram of extract.

Table 2. Evaluation of antioxidant activity of crude extract and leaf partition phases of the four genotypes of *Feijoa sellowiana* and the wild specimens (control). All values are expressed in mg TE/g of extract.

Sample	Genotypes				Wild (control)
	Alcântara	Helena	Mattos	Nonante	
CE	264.19 ± 2.77	166.79 ± 5.88	177.84 ± 9.11	233.12 ± 10.10	262.13 ± 3.71
Hx	32.73 ± 1.61	57.48 ± 1.30	64.27 ± 1.17	11.64 ± 1.61	15.23 ± 0.46
DCM	91.81± 2.83	73.98 ± 5.59	66.19 ± 1.58	85.29 ± 1.80	83.49 ± 2.71
AcOEt	269.98 ± 0.53	268.92 ± 0.42	269.18 ± 0.35	267.85 ± 0.58	269.45 ± 0.23
BuOH	267.85± 0.81	268.12 ± 0.42	269.12 ± 0.81	266.86 ± 1.67	268.32 ± 0.46
HA	229.27± 1.92	162.67 ± 13.31	230.26 ± 7.45	235.32 ± 3.03	172.91 ± 5.36

CE: crude extract; Hx: hexane partition phase; DCM: dichloromethane phase; AcOEt: ethyl acetate; BuOH: butanol phase; HA: residual hydroalcoholic phase.

Saber (2021) achieved promising antioxidant results with the specimens available in Egypt, with values of 90.58 mg TE/g leaves extract. Compounds such as α-tocopherol and quercetin and its glucoside avicularin were associated with this activity. Burbano-Ipiales (2022) reported 16.72 mg ± 0.15 TE/g FW (Fresh weight) for the epicarp of feijoa, while Amarante (2017) reported 3.27 mg/g FW. The difference in antioxidant activities of different parts of the plant can be primarily attributed to the variation in the content of total phenolics and flavonoids in each of them (Feduraev et al., 2019; Okello et al., 2021).

Comparing the chromatograms (Figure 1) of the crude extract with those of the partition phases that showed higher antioxidant activity (ethyl acetate and butanol), it is observed that most of the compounds present in the crude extract are preserved, although the intensity of the peaks varies. Quercetin stands out as the most abundant compound in these phases, followed by rutin. A peak

related to ellagic acid, which was not detected in the crude extract, appears in these fractions. This compound was already reported by Aoyama (2018) and Cebi & Sagdic (2022) in leaf extracts. Chromatograms of the Mattos genotype was selected as reference, as they present all the compounds initially identified in the crude extract.

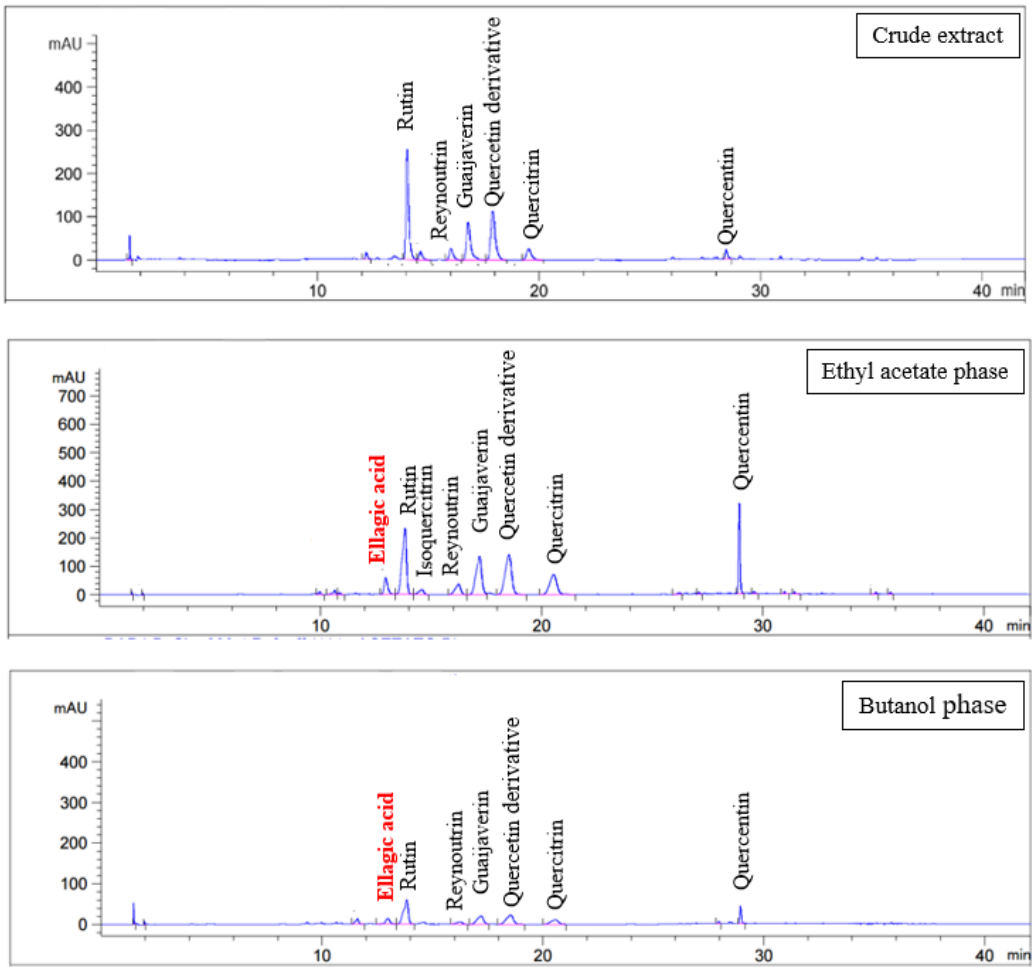

Figure 1. Chromatograms of the Mattos genotype obtained by HPLC-DAD analysis, at a wavelength of 352 nm of the crude extract and the phases in ethyl acetate and butanol obtained from the leaf extract of *Feijoa sellowiana*. The x-axis corresponds to the retention time (min) of each peak and the y-axis corresponds to the intensity of the peaks in mAU.

Flavonoids are recognized as effective antioxidants, and their chemical structure is related to their activity (Heim et al., 2002; Pérez-Trueba, 2003). Quercetin, the most potent antioxidant found in nature, has been associated with the prevention of diseases such as osteoporosis, certain types of cancer, as well as pulmonary and cardiovascular diseases (Lakhanpal et al., 2007; Xu et al., 2019; Pratyusha et al., 2022). In addition, it possesses antibacterial, antifungal, and anti-inflammatory action (Azeem et al., 2023). It acts on glutathione (GSH), enzyme activity, signal transduction pathways and reactive oxygen species (ROS) (Xu et al., 2019). Rutin has shown activity against several types of cancer and is considered one of the most potent antioxidants (Satari et al., 2021). In addition, vasoprotective, neuroprotective and cardioprotective properties have been also attributed to this compound (Ganeshpurkar et al., 2017). On the other hand, guajaverin has been shown to improve pancreatic β-cell function and hepatocyte morphology in diabetic mice (Kumar et al., 2021).

4. Conclusions

The preliminary results of this study indicate that there are no statistically significant differences in total phenol content between Brazilian genotypes Alcântara, Helena, Mattos, and Nonante, developed by EPAGRI, and wild specimens of *Feijoa sellowiana*. The analysis revealed that compounds derived from quercetin predominated in all groups. However, further analysis is required for the tentative identification of the other compounds. Additionally, the extracts exhibited significant antioxidant activity, especially in the ethyl acetate and butanol partition fractions. The predominant presence of compounds such as quercetin and rutin highlights the importance of these flavonoids in the antioxidant activity of the extracts. These findings confirm the phenolic richness and antioxidant capacity of *Feijoa sellowiana* leaves,

highlighting their potential use in therapeutic and nutraceutical applications. Furthermore, the results underscore the importance of optimizing extraction methods to maximize the yield of these bioactive compounds.

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General discussion and conclusion

The continuous research of the species not only allows us to expand our knowledge, but also brings us closer to the identification and development of new molecules with potential applications in various industries. The four genotypes of *Feijoa sellowiana* developed by EPAGRI, Alcântara, Helena, Mattos and Nonante, are some of the solutions through which we seek to expand the knowledge of the species in Brazil, as well as to improve the production and quality of its fruits.

The breeding process for these four genotypes was carried out through traditional techniques involving mostly Brazilian accesses, except for Helena in which the genotype "Unique" from New Zealand was used. They were released in 2007 – 2008, and the main difference between the four genotypes lies in the ripening period, which allows producers to carry out a staggered harvest.

The chemical profiles of the Brazilian genotypes of *Feijoa sellowiana*, as well as the wild species used as a control, showed a wide diversity of secondary metabolites. However, both similarities and notable differences were found between the profiles. Cuticular waxes stood out for the predominant presence of long-chain hydrocarbons, with fatty acids and primary alcohols as predominant classes. Oxygenated and non-oxigenated monoterpenes and sesquiterpenes represented more than 94% of the leaf essential oil profiles. Among phenolic compounds, glycosides of quercetin and it derivatives were common.

Mattos and Alcântara were the most differentiated genotypes. Mattos showed a predominance of primary alcohols in the cuticular waxes and a higher percentage of monoterpenes in the volatile oils compared to the other groups, in addition to having the most diverse phenolic profile in the crude extract. Alcântara, on the other hand, was characterized by a predominance of fatty acids in the cuticular waxes and presented the highest percentages of sesquiterpenes (90.6%) in the volatile oils. As for phenolic compounds, although it was the least diverse genotype, the major compounds such as rutin, guajaverin and a derivative of quercetin, present in other groups, were also found in Alcântara. Helena, Nonate and the control group (wild) showed similar profiles of cuticular waxes and volatile oils (Figure 2 - Chapter 2; Figure 4 - Chapter 4). As for phenolic compounds, preliminary data did not reveal substantial differences between these groups.

In summary, the Brazilian genotypes of *Feijoa sellowiana* represent a valuable resource in the research and development of this species in Brazil. The chemical diversity among these genotypes, represented in their profiles of cuticular waxes, volatile oils, and phenolic compounds, offers a wide range of possibilities for their application in various industries. We hope that this study will contribute not only to scientific knowledge, but also to the genetic improvement and optimization of Feijoa production in Brazil.

Resumo geral

A Feijoa sellowiana é uma espécie pertencente às Myrtaceae. Embora seja nativa do sul do Brasil, apresenta desafios em sua domesticação devido a doenças, fatores genéticos e ambientais. No entanto, países como a Colômbia e a Nova Zelândia conseguiram estabelecer cultivos eficientes, sendo o fruto a parte mais utilizada, com ampla aplicação na indústria alimentícia. A Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina - EPAGRI desenvolveu os genótipos Alcântara, Helena, Mattos e Nonante, adaptados a diferentes ambientes, com resistência a pragas e doenças, rendimentos consistentemente altos e frutos de tamanho uniforme e bom sabor. O presente estudo teve como objetivo avaliar e comparar o perfil químico dos extratos de folhas desses genótipos, juntamente com espécimens selvagens, para determinar se o processo de melhoramento afetou seus perfis químicos. Foram avaliados os lipídios de superfície, os compostos de óleo volátil e os fenólicos, bem como a atividade antioxidante em extratos brutos e fases de partição. Os resultados revelaram uma ampla diversidade de metabólitos secundários, juntamente com uma atividade antioxidante significativa. A extração de ceras cuticulares foi realizada por imersão repetida em diclorometano, seguida de análise por cromatografia gasosa acoplada à espectrometria de massa (CG-EM). Os ácidos graxos e os álcoois primários foram as classes químicas principais nas ceras em todos os grupos, embora o ácido ursólico e o 1-hexacosanol tenham sido os compostos predominantes. Foi observada maior variação nos perfis químicos da cera nos genótipos Mattos e Alcântara. Com relação aos componentes dos óleos voláteis obtidos por destilação e analisados por CG-EM, os sesquiterpenos e monoterpenos foram as classes mais proeminentes, sendo o biciclogermacreno, o germacreno D e o cariofileno os compostos mais

abundantes. O genótipo Mattos foi notado por seu alto teor de monoterpenos em comparação com os outros grupos. Já os compostos fenólicos foram analisados por cromatografia líquida de alta eficiência com detector por arranjo de diodo (CLAE- DAD, revelando um teor de compostos fenólicos totais nas folhas que variou de 10,33 ± 4,09 a 13,70 ± 5,35 mg de equivalente de ácido gálico por grama de extrato, sem diferenças estatisticamente significativas entre os grupos. As fases de partição de acetato de etila e de butanol apresentaram considerável atividade antioxidante, principalmente devido à presença de rutina e quercetina. Em conclusão, o estudo fornece uma visão inicial da composição química das folhas dos genótipos brasileiros de Feijoa sellowiana, destacando sua diversidade e potencial para diversas aplicações. O processo de melhoramento realizado em Mattos e Alcântara parece ter influenciado o metabolismo secundário desses dois genótipos, uma vez que a composição das ceras e dos óleos voláteis os diferenciam dos demais. Essas descobertas podem aumentar o interesse econômico e a pesquisa acadêmica sobre a espécie, além de serem úteis na seleção de variedades.

Palavras-chave: Feijoa sellowiana, genótipos brasileiros, composição química, antioxidante

General abstract

Feijoa sellowiana is a species belonging to the Myrtaceae. Although it is native to southern Brazil, this species presents challenges with its domestication due to diseases, genetic and environmental factors. However, countries such as Colombia and New Zealand have succeeded in establishing efficient crops, with the fruit being the most widely used part with application in the food industry. The Agricultural Research and Rural Extension of Santa Catarina - EPAGRI developed the genotypes Alcântara, Helena, Mattos and Nonante that are adapted to different environments, with resistance to pests and diseases, consistently high yields and fruits of uniform size and good flavor. The present study focused on evaluating and comparing the chemical profile of leaf extracts of these genotypes, together with the wild specimens, to determine if the breeding process had affected their chemical profiles. Surface lipids, essential oil compounds and phenolics were evaluated, in addition to antioxidant activity in crude extracts and partition phases. The results revealed a wide diversity of secondary metabolites along with significant antioxidant activity. Extraction of cuticular waxes was performed by repeated immersions in dichloromethane, followed by analysis by gas chromatography coupled to mass spectrometry (GC-MS). Fatty acids and primary alcohols were found to be the most common chemical classes of wax components in all groups, although ursolic acid and 1-hexacosanol were the most prevalent compounds. Greater variation in wax chemical profiles was observed in the Mattos and Alcântara genotypes. In relation to the components of the essential oils obtained by distillation and analyzed by GC-MS, it was found that sesquiterpenes and monoterpenes were the most prominent classes, with bicyclogermacrene, germacrene D and

caryophyllene being the most abundant compounds. The Mattos genotype was highlighted for its high content of monoterpenes compared to the other groups. Phenolic compounds were analyzed by high performance liquid chromatography with diode-array detection (HPLC-DAD), revealing a total phenol content in leaves ranging from 10.33 ± 4.09 to 13.70 ± 5.35 mg of gallic acid equivalent per gram of extract, with no statistically significant differences between groups. The ethyl acetate and butanol partition phases exhibited considerable antioxidant activity, mainly due to the presence of rutin and quercetin. In conclusion, the study provides an initial insight into the variation of chemical composition of the leaves of Brazilian genotypes of *Feijoa sellowiana*, highlighting their diversity and potential for diverse applications. Breeding process carried out in Mattos and Alcântara seems to influence the secondary metabolism of these two genotypes, once their wax and essential oil profiles put them apart from the others. These findings could boost both economic interest and academic research on the species, in addition to being useful in varietal selection.

Key words: *Feijoa sellowiana*, brazilian genotypes, chemical composition, antioxidant