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Secondary components in *Eugenia* (Myrtaceae): Extraction and Efficiency as an antimicrobial in *E. francavilleana* and *E. paracatuana*

Componentes secundários em *Eugenia* (Myrtaceae): Extração e Eficiência como antimicrobiano em *E. francavilleana* e *E. paracatuana*

Componentes secundarios en *Eugenia* (Myrtaceae): Extracción y Eficiencia como antimicrobiano en *E. francavilleana y E. paracatuana*

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Abstract
Organic extractives (OrgExt), also known as a secondary component of plants, have attracted interest of the scientific community in recent years due to their low toxicity and remarkable antimicrobial activity. This present work has aimed to increase knowledge about OrgExt of two Myrtaceae species of Brazilian flora: *Eugenia francavilleana* and *E. paracatuana*. OrgExtr of selected species were extracted by two different organic solvents to compare their efficiency and yieldness, namely: cylohexane/ethanol and hexane. Paired T statistical method was used to evaluate yield of extraction process for both species. Quantitative determination of the main compounds presented in structures of plant tissues was performed (Klason lignin content, α-cellulose, holocellulose and polyoses). Inorganic components presented in ash of species were determined by EDS. Among organic solvents tested for extraction of essential substances, the most efficient was hexane, being *Eugenia francavilleana* the species that provided the highest yield of extracted oil. OrgExt demonstrated simultaneous efficiency in substitution of glycerol (plasticizer) in preparation of thermoplastic starch films and as antimicrobial agents, delaying and reducing appearance of microorganisms in these films. This study has increased knowledge of two species whose OrgExt had never been studied before and demonstrated a potential application.
Keywords: Organic extractives; Plasticizing agents; Antimicrobial properties; Essential oils; Myrtaceae; Leaves.

Resumo
Extrativos Orgânicos (OrgExt), também conhecidos como componentes secundários de plantas, têm despertado o interesse da comunidade científica nos últimos anos devido à sua baixa toxicidade e notável atividade antimicrobiana. O presente trabalho teve como objetivo aumentar o conhecimento sobre OrgExt de duas espécies de Myrtaceae da flora brasileira: *Eugenia francavilleana* e *E. paracatuana*. OrgExt das espécies selecionadas foram extraídas por dois solventes orgânicos diferentes para comparar sua eficiência e rendimento, a saber: ciclohexano/etanol e hexano. O método estatístico T emparelhado foi usado para avaliar o rendimento do processo de extração para ambas as

espécies. Foi realizada a determinação quantitativa dos principais compostos presentes nas estruturas dos tecidos vegetais (teor de lignina Klason, α-celulose, holocelulose e polioses). Os componentes inorgânicos presentes nas cinzas das espécies foram determinados por EDS. Dentre os solventes orgânicos testados para extração de substâncias essenciais, o mais eficiente foi o hexano, sendo *Eugenia francavilleana* a espécie que proporcionou maior rendimento de óleo extraído. OrgExt demonstrou eficiência simultânea na substituição do glicerol (plastificante) na preparação de filmes de amido termoplástico e como agente antimicrobiano, retardando e reduzindo o aparecimento de microrganismos nestes filmes. Este estudo aumentou o conhecimento de duas espécies cujo OrgExt nunca havia sido estudado antes e demonstrou uma aplicação potencial.
Palavras-chave: Extrativos orgânicos; Agentes plastificantes; Propriedades antimicrobianas; Óleos essenciais; Myrtaceae; Folhas.

Resumen
Los extractivos orgánicos (OrgExt), también conocidos como componente secundario de las plantas, han atraído el interés de la comunidad científica en los últimos años debido a su baja toxicidad y notable actividad antimicrobiana. El presente trabajo tuvo como objetivo aumentar el conocimiento sobre OrgExt de dos especies de Myrtaceae de la flora brasileña: *Eugenia francavilleana* y *E. paracatuana*. OrgExt de especies seleccionadas se extrajeron con dos solventes orgánicos diferentes para comparar su eficiencia y rendimiento, a saber: ciclohexano/etanol y hexano. Se utilizó el método estadístico T pareada para evaluar el rendimiento del proceso de extracción para ambas especies. Se realizó la determinación cuantitativa de los principales compuestos presentes en las estructuras de los tejidos vegetales (contenido de lignina Klason, α-celulosa, holocelulosa y poliosas). Los componentes inorgánicos presentes en las cenizas de las especies se determinaron por EDS. Entre los solventes orgánicos probados para la extracción de sustancias esenciales, el más eficiente fue el hexano, siendo *Eugenia francavilleana* la especie que proporcionó el mayor rendimiento de aceite extraído. OrgExt demostró eficiencia simultánea en sustitución de glicerol (plastificante) en la preparación de películas termoplásticas de almidón y como agente antimicrobiano, retrasando y reduciendo la aparición de microorganismos en estas películas. Este estudio ha aumentado el conocimiento de dos especies cuyo OrgExt nunca se había estudiado antes y demostró una aplicación potencial.
Palabras clave: Extractivos Orgánicos; Agentes plastificantes; Propiedades antimicrobianas; Aceites esenciales; Myrtaceae; Folhas.

1. Introduction

Medicinal plants have been used since antiquity as medicines, due to their ability to synthesize a wide variety of chemical compounds that have been used to perform important biological functions and to defend against attack of predators, such as insects, fungi, herbivores and mammals (R. F. da Silva et al., 2022). Preliminary studies indicated that many species of Myrtaceae family have had antimicrobial properties. Among *Eugenia* species we could mention: *Eugenia pyriformis* that inhibited growth of bacteria and fungi (Souza et al., 2011); *Eugenia uniflora* rich in flavonoids, used to control hypertension and gout (Oliveira et al., 2005); *Eugenia stipitata* and *Eugenia dysenterica* with antimicrobial potential (Oliveira et al., 2005); *Eugenia punicifolia* used in treatments of wounds and infectious diseases (Oliveira et al., 2005).

Brazil is a country rich in plant diversity with a higher rate of endemism, essential for strategic biotechnology industry, in addition to presenting a great economic potential (de Paulo Farias et al., 2020). Myrtaceae is a pantropical family with ca. 6000 species distributed through Australasia and the Pacific, tropical and subtropical America, and with a smaller African representation (Lucas et al., 2019; *Royal Botanic Gardens*, 2022). *Eugenia* is the largest genus of Neotropical Myrtaceae; with about 1000 species (407 native in Brazil (Mazine et al., 2020)), and it is the most species-rich angiosperm genus in Brazil (Mazine et al., 2020). According to Souza (Souza et al., 2011), Organic extractives (OrgExt) were found in forty-eight species from subtribe *Eugeniinae* (which besides *Eugenia* also includes genus *Myrcianthes* (Mazine et al., 2014, 2018)), which has presented a higher proportion of sesquiterpenes than monoterpenes.

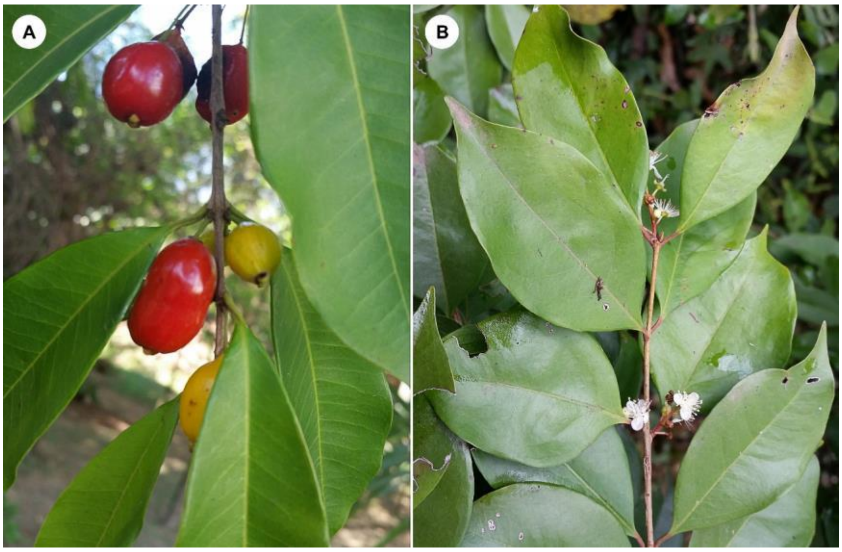
OrgExt have raised scientific community's interest in recent years due to their low toxicity and remarkable antimicrobial and antioxidant activity (Tariq et al., 2019). OrgExt could be extracted from leaves, flowers, bark, fruits, and rhizomes, depending on which species were being explored. To perform extraction there were several methods, such as steam-dragging technique, cold pressing, hydrodistillation, solvent extraction, and supercritical fluids (Bizzo et al., 2009). Solvent extraction process consisted of placing an organic solvent in contact with plant matrix. After a period of time that was sufficient to transfer the soluble constituents presented in the plant, solid and liquid phases were separated. And so, extract was obtained by evaporation of solvent presented in liquid phase (Bizzo et al., 2009).

According to Botaro and Rodrigues (Botaro & Rodrigues, 2019), chemical composition of lignocellulosic materials was formed by fundamental components (cellulose, lignin and hemicellulose), which were part of structural wall of wood and secondary or accidental components that were not part of formation of cell wall or middle lamella. Secondary components were substances of low and medium molar mass and were classified in two groups: organic natures (extractives) and inorganic substances (ashes). Extractives included a large number of compounds, such as: phenols, terpenes, lignin, steroids, acid resins, resins, waxes, greases, fatty acids, fatty alcohols, essential oils and other types of organic compounds (Botaro & Rodrigues, 2019).

To make sustainable management of forest, one option was exploitation of non-timber forest products and their extractives. Non-timber forest products allowed management to be community-based, with a greater engagement of people, a subsistence component, and besides valuing the forest that is preserved standing (Kumar et al., 2009). OrgExt were few studied and through them, solutions could be obtained for several problems, such as control of fungi, bacteria and insects. Its representation could be very valuable in cosmetic industries due to its use as an essence for perfumes and creams (Botaro & Rodrigues, 2019).

This present work deepened knowledge about OrgExt of two species of Myrtaceae, *Eugenia francavilleana* and *E. paracatuana* (Figure 1), using solvent extraction technique. Paired T-statistical method was employed to evaluate yield of extraction process for both species. Quantitative determination of main compounds presented in plant tissue structures (Klason lignin content, α-cellulose, holocellulose and polyoses) was performed. Inorganic components presented in ashes of species were ascertained by EDS. Finally, OrgExt produced were employed in synthesis of easily accessible and low-cost films, as an antimicrobial agent and as a substitute for commercial glycerol, to produce thermoplastic cornstarch films. Through incorporation of extracts, it was possible to identify antimicrobial action comparatively and visually.

Figure 1. Fresh reproductive branches of (A) *Eugenia francavilleana*, with mature and immature fruits, and (B) *Eugenia paracatuana*, with flowers.



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2. Methodology

2.1 Materials

Cyclohexane PA (99.66 %), ethyl alcohol (99.8 %) and hexane PA were supplied by Synth®. Starch used was AMIDEX 3001 (lot: 70335) supplied by Daxia® (Characteristics - Maximum humidity: 14%; pH: 4.5 - 5.5) and Glycerin P.A. (Glycerol) from Dinamica®.

2.2 Plant origin

Species *Eugenia francavilleana* (Figure 1A) and *E. paracatuana* (Figure 1B) used in this project were collected in two forest fragments: in Condomínio Fazenda Jequitibá, in municipality of Sorocaba/SP, Brazil, and in Mata do Godinho, in municipality of Piracicaba/SP, Brazil, respectively. Leaves and branches were transported to the Federal University of São Carlos (UFSCar) - *campus* Sorocaba and dried in a 60 ºC oven at the Herbarium of the Science and Technology Center for Sustainability - UFSCar *campus* Sorocaba. After drying, vouchers of both species (exsiccata) were deposited in SORO Herbarium for further consultation. *Eugenia francavilleana* and *E. paracatuana* are native species of Brazilian flora and occur in the region where the study was developed, which facilitated their collection (A. T. Da Silva & Mazine, 2016; Mazine et al., 2020).

2.3 Removal of water-soluble extractives

Removal and calculation of water-soluble extractives content were determined by ASTM D1110-84 (American Society for Testing and Materials (D1110-84), 2013). In this procedure, about 30 g of *Eugenia paracatuana* and *E. francavilleana* leaves were weighed *in nature*. Then, in a beaker with 1 L of distilled water was added to each sample previously weighed and placed under a hot plate at 70 ºC (± 5 ºC) with magnetic stirring for one hour. Operation was repeated changing water. After water extraction period, mixture went through vacuum filtration with a Buchner funnel. Sample retained on filter paper was dried in an oven at 60 ºC until it completely dried and then placed in a desiccator until it reached room temperature. Then it was weighed on an analytical balance. Whole procedure was performed in triplicate to calculate water-soluble extractive content.

2.4 Organic extractives

Dry fibers, free of water-soluble extractives, were weighed and packed in filter paper cartridges to extract soluble extractives in organic solvents through Soxhlet extractor, using 350 mL of a mixture of cyclohexane/ethanol (1:1, vol.), and same process was performed for hexane. Extraction time was 3 hours, and was carried out until mixture of solvents passing through cartridge did not present color inside iphon of the system. After removing filter paper cartridges, they were dried at 70 ºC in an oven and then weighed on an analytical balance to calculate the soluble extractive content in organic solvents presented leaves. Content of extractives soluble in organic solvents was determined by TAPPI T207 cm-93 (Technical Association of Pulp and Paper Industry (TAPPi T204 cm-97), 1998).

2.4.1 Yield

Yield of process was calculated based on initial mass of dry, pre-treated fiber and final mass of OrgExt according to Equation 1.

$$Yield = \frac{m1 - m2}{m1} x100 \qquad \text{Equation 1}$$

Where: m1= mass of dried leaves after extraction process (g); m2= initial mass of dried leaves (g); Yield= Yield of extractives soluble in organic solvents (%).

Based on data obtained from six replicates of extractions, paired t statistical method was performed with independent samples to verify whether yields of extractions differed significantly or not. Analyses were performed in software R. Statistical and conclusion was made from data interpretation, mainly the data p_value that quantified discrepancy between data and a null hypothesis of interest. In this work, null hypothesis was that there was no statistical difference between compared data. paired T statistical test, with independent samples, was used to compare differences between solvents for both species investigated.

2.5 Characterization of the secondary components

Moisture content of leaves, previously dried and ground, was determined according to ABNT NBR 1423:2017 (Associação Brasileira de Normas Técnicas (NBR 1423:2017), 2017), and it was checked before and after each analysis and/or chemical process to obtain the real value of sheet used in each process, in grams, without percentage of water presented in material. Ash content, klason lignin content, α-cellulose content, and holocellulose content were performed according to ASTM D1102-84 (American Society for Testing and Materials (D1102-84), 2013), ASTM D1106-96 (American Society for Testing and Materials (D1106-96), 2013), ASTM D1103-60 ((D1103-60), 1985) e ASTM D1104-56(American Society for Testing and Material (D1104-56), 1985), respectively. Polyoses content was calculated by subtracting holocellulose and α-cellulose content.

2.6 Fourier Transform Infrared Spectroscopy (FTIR)

Structural characterization was performed in Lignocellulosic Materials Laboratory of the UFSCar – *campus* Sorocaba. Equipment used was Nicolet summit IR 200 FT-IR model in % reflectance mode, using 126.0 scans, nominal resolution of 4.0 cm^{-1}, in range 4000 to 400 cm^{-1}. Spectra were obtained in Ominic Paradigm (Thermo Scientific, USA) and by means of Origin Pro 8.0 the spectra were also obtained.

2.7 Energy dispersive X-ray spectroscopy (EDS)

EDS (Energy Dispersive System), also known as energy dispersive X-ray analysis, is a system coupled to Scanning Electron Microscope (Hitachi, model TM3000), which from emission of characteristic X-rays determines semiquantitatively chemical composition of sample under study. Samples were fixed on metal support on double-sided carbon tape, with an applied acceleration voltage of 15 kV. Peaks ≥ 0.4 cps/ev (counts per second per electron volt) were considered throughout spectrum from 0 to 10 keV (kilo electron volt). Ash of *Eugenia francavilleana* and *E. paracatuana* were subjected to EDS analysis to detect inorganic components.

2.8 Film Preparation

For evaluation of antifungal property of OrgExt of different species, five different types of film were prepared: i) starch film without OrgExt (Control); ii) starch film with OrgExt from *Eugenia francavilleana* extracted with cyclohexane/ethanol (Fra Ciclo); iii) starch film with OrgExt from *E. francavilleana* extracted with hexane (Fra Hex); iv) starch film with OrgExt of *E. paracatuana* extracted with cyclohexane/ethanol (Para Ciclo); v) starch film with OrgExt of *E. paracatuana* extracted with hexane (Para Hex). Such films were obtained using 2 g of corn starch, 1 g of glycerol, and 17 mL of distilled water (Control), and for other films, 1 g of OrgExt was added in place of glycerol.

Subsequently, beaker with constituents of each film was placed on a heating board, under stirring, until it reached a temperature of approximately 80 ºC, and kept at this temperature for 5 minutes for starch gelatinization. After this period, the mixture had a viscous aspect and was then poured into circular plastic containers for drying at room temperature (25 ºC) and for formation of films. Plastic containers remained open and were kept in the chapel during the total period of 15 days for a comparative and visual analysis of antimicrobial action of different films formulated.

3. Results and Discussion

3.1 Yield of Organic extractives

Yield of extraction process ranged from 0.72 % to 1.42 % for *Eugenia paracatuana* extracted respectively by solvent hexane and cyclohexane/ethanol. For *E. francavilleana*, yield ranged from 0.91 % to 1.69 % extracted by hexane and cyclohexane/ethanol solvent, respectively. In work of Azevedo [16] yields of 0.02 % and 1.00 % were observed for *E. feijoi* and *E. pseudopsidium*, respectively (Azevedo, 2014). Which made it clear that results presented here were much more promising.

Statistical analyses of process yield were performed from a script prepared in statistical software R. Comparing data of solvents in extraction of OrgExt from *Eugenia francavilleana* and *E. paracatuana*, presented a p_value less than 0.05 indicating that results differed at 5.00 % significance, i.e., chance of statistical test being incorrect was 5.00 %, so there was a 95.00 % chance of statistical result being correct. Thus, it could be concluded that cyclohexane/ethanol mixture extracted more compounds than hexane solvent, thus presenting a higher yield. Result was expected due to being a mixture, where cyclohexane was nonpolar and ethanol polar; with junction of two solvents with different polarities, it was expected to have a greater ability to remove different molecules, both polar and nonpolar. Despite being an nonpolar solvent, hexane showed lower yield due to less contact of hydrocarbon molecules, resulting in less polarization than observed in cyclohexane. Besides being a very specific solvent for some molecules presented in plant tissues, limiting extraction of polar molecules that ethanol could remove (Carvalho et al., 2014).

3.2 Inorganic substances and moisture content

Ash content of species *Eugenia francavilleana* and *E. paracatuana* were determined by residue content of complete leaf burning, according to ASTM D1102-84 (Standard Test Method for Ash in Wood (D1102-84), 2013). Values found were presented and compared in Table 1.

Table 1 - Moisture and ash content of *Eugenia paracatuana* and *E. francavilleana*.

Components	Experimental result (%)		Comparison (%)		
	E. paracatuana	*E. francavilleana*	*E. dysenterica* (Couto et al., 2009)	*E. uniflora* (N. M. Rodrigues et al., 2010)	*E. brasiliensis* (Nehring, 2016)
Ash Content	5,35±0,27	7,30±0,39	2,94±0,073	8,40±1,32	3,61±0,8
Moisture	3,77±0,45	11,60±0,53	8,15±0,132	from 8 to 14	7,43±1,70

Source: Authors.

Ash content, on average, for both species was higher than what we have found in literature for other species of *Eugenia*. Percentage of ash found was 5.35 % ±0.27 for *Eugenia paracatuana* and 7.30 % ±0.39 for *E. francavilleana*. Values found in literature were 2.94 % ±0.07 for *E. dysenterica* (Couto et al., 2009), 8.40 % ±1.32 for *E. uniflora* (N. M. Rodrigues et al., 2010) and 3.61 % ±0.8 for *E. brasiliensis* (Nehring, 2016). Increase in ash content of species studied was possible due to growing region that had a higher concentration of calcium and magnesium in its soil. Humidity found for the species *E. paracatuana* was lower than what was found in literature, being 3.77 % ±0.45, while humidity found for *E. francavilleana* was within values observed in literature, being 11.60 % ±0.53. Values found in literature were 8.15 % ±0.13 for *E. dysenteric*a (Couto et al., 2009), 8 to 14 for *E. uniflora* (N. M. Rodrigues et al., 2010), and 7.43 ± 1.70 for *E. brasiliensis* (Nehring, 2016).

3.2.1 Chemical composition analysis by EDS

Ashes of *Eugenia francavilleana* and *E. paracatuana* were submitted to EDS analysis to detect inorganic components (Table 2). Main elements found in ashes of *E. francavilleana* were calcium, oxygen and magnesium, being also found in smaller amounts phosphorus, sulfur, among others. And main elements found in ashes of *E. paracatuana* were calcium, oxygen, silicon, aluminum, potassium, sulfur, and, to a lesser extent, magnesium, phosphorus, carbon, sodium, among others.

Table 2 - EDS of the ashes of *Eugenia francavilleana* and *E. paracatuana*.

Chemical elements	Percentage (%) *E. francavilleana*	Percentage (%) *E. paracatuana*
Calcium	57,2	41,5
Magnesium	5,3	2,2
Phosphorus	1,5	2,7
Arsenic	1,1	-
Sulfur	0,8	3,1
Aluminum	0,7	6,1
Silicon	-	6,4
Potassium	-	3,3

Source: Authors.

Both calcium and oxygen were found in larger amounts for both species analyzed. Calcium is an element that performs several functions in plants, mainly in protection of membranes and cell walls and in signaling response to biotic or abiotic stress conditions (Yamamoto, 2011). On the other hand, oxygen is used in respiration and nitrogen metabolism (Antônio et al., 2002). Magnesium found in large amounts in *Eugenia francavilleana* is an activator of several enzymes related to synthesis of carbohydrates and nucleic acids (Sfredo & Borket, 2004) is in lower concentration in *E. paracatuana*, signaling a possible deficiency of it.

In *Eugenia paracatuana* aluminum was found in high concentration if compared to *E. francavilleana*, this fact may have occurred because the soil of collected specimens was acidic. Aluminum is a toxic element and may be responsible for low productivity and constitute a limiting factor to plant growth (Couto et al., 2009). Another element found in *E. paracatuana* was potassium, which is a highly mobile nutrient, acting in enzyme activation, regulating opening and closing of stomata, acting in osmotic tissue regulation, and increasing plant resistance to diseases (Sfredo & Borket, 2004). *Eugenia francavilleana* showed a low concentration of sulfur, which is an element presented from ionic uptake to RNA and DNA roles to hormonal control for cell growth and differentiation (Stipp & Casarin, 2010), signaling a deficiency of this nutrient necessary for plant growth.

3.3 Analysis of content of insoluble lignin Klason, α-cellulse, holocellulose and polyoses.

In chemical composition of wood is lignin, which is responsible for transporting nutrients and water within vascular structure and provides support and resistance (J. S. Rodrigues et al., 2020). Cellulose and polyoses are responsible for sustaining cell walls (Botaro & Rodrigues, 2019). Experimental results and comparison made with literature have been presented in Table 3.

Table 3 - Chemical composition of *Eugenia paracatuana* and *E. francavilleana*.

Components	**Experimental result (%)**		**Comparison (%)**	
Species	*Eugenia paracatuana*	*Eugenia francavilleana*	*Eucalyptus* chips	*Eucalyptus grandis x urophylla*
Klason Lignin	41,85±2,22	50,90±2,28	34,54±3,4	21,72
α-cellulose	40,50±4,95	32,00±2,36	40,88±0,1	49,04
Holocellulose	63,31±0,48	52,50±2,37	55,70±0,1	71,15
Polyose	23,30±2,72	20,50±2,37	14,82±0,1	22,11

Source: Authors.

Chemical composition was determined according to quantitative analysis, species *Eugenia paracatuana* showed a lignin content of 41.85 % (Table 3), on a dry basis. This result is above average values found in literature of 34.54 % (Filho, 2016) and 21.72 % (Hsing et al., 2016). Same was done with species *E. francavilleana* which showed a lignin content of 50.90 % (Table 3), on a dry basis. High lignin content in studied species could be explained by presence of bark, lignin-rich component, mixed with foliage. Comparing contents of both species studied, we noticed that *E. francavilleana* had a higher percentage of lignin content, therefore a more lignified wood, with greater hardness. This variation in lignin content in plants may have occurred due to several factors, such as micronutrients, water availability, climate, and other factors. Another possible factor is that growth of species was different, as well as their density. Wood from *Eugenia paracatuana* and *E. francavilleana* had a higher percentage of lignin and was therefore denser.

Cellulose was a carbohydrate formed by condensed glucose units joined by glycosidic bonds (Botaro & Rodrigues, 2019). Content of α-cellulose of species *Eugenia paracatuana* was as expected according to literature, with a content of 40.50 % compared to 40.88 % (Filho, 2016) and 49.04 % (Hsing et al., 2016). The α-cellulose content of species *E. francavilleana* was lower than expected, with a content of 32.00 % (Table 3), this fact may be related to higher percentage of lignin in this species. Content of α-cellulose could vary between species, even if they were from same family and group, depending on several factors, such as region where it was planted and climatic conditions.

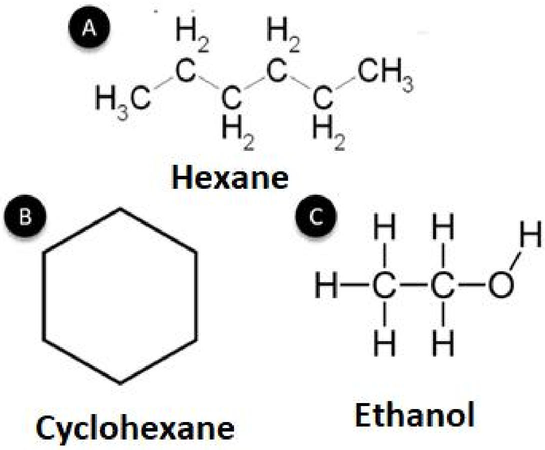
Polyoses are sugar polymers with five carbons (Fernando et al., 2006). Species *Eugenia paracatuana* showed polyoses content of 23.30 % (Table 3) and species *E. francavilleana* showed polyoses content of 20.50 %, both species showed values compatible with literature, being comparative contents of 22.11 % (Hsing et al., 2016) and 14.82 % (Filho, 2016).
Holocellulose was the sum of α-cellulose and polyoses, so these were carbohydrates presented in samples of both species. Holocellulose content of *Eugenia paracatuana* was 63.31 % (Table 3) and that of *E. francavillean*a was 52.50 %, experimental values were similar to values found in literature, which were 71.15 % (Hsing et al., 2016) and 55.7 % (Filho, 2016). Holocellulose content could vary between species, even if they were from same group and family, depending on factors such as: region where it was planted and climatic conditions.

After analysis of Table 3, it was found that, in general, results obtained in chemical analyses were similar to those found in literature for other species of *Eugenia*, and may present some variations related to various factors presented during growth of individuals.

3.4 Extractives

Extractives had a wide distribution and could be classified as: aliphatic, nitrogenous, aliphatic, terpenes, steroids, and glycosides (Botaro & Rodrigues, 2019). Wood furthest away from vascular cambium tended to present greater contractions in total extractives. Proportions of extractives were found in significant amounts in only some species or genera and still extractives could occur in various plant organs. Almost always, amounts in these parts of trees were proportionally greater than in wood (Botaro & Rodrigues, 2019). Presence of extractives influenced resistance to attack by fungi and insects, color, odor, permeability, wood hardness, and density (Moreira et al., 2016). Extractive content was an important indicator of wood compliance for various industrial uses (Botaro & Rodrigues, 2019). Choice of organic solvents was based on their efficiency in extracting polar and non-polar molecules. Organic solvents: Hexane and cyclohexane/ethanol (Figure 2) were used to extract the OrgExtr.

Figure 2 - Chemical structure of the compounds (A) Hexane, (B) Cyclohexane and (C) Ethanol.



Source: Authors.

In Figure 2 A, solvent molecule hexane was represented, an alkane hydrocarbon with a single bond between carbon and hydrogen. Cyclohexane (Figure 2 B) was composed of carbon, hydrogen, and aromatic ring, having a single bond between carbon and hydrogen and a double bond. Ethanol, as seen in Figure 2 C, was formed by two carbon atoms bonded to five hydrogen atoms and to an oxygen atom bonded to another hydrogen atom. Presence of hydroxyl (OH) made this molecule polar, and bond was simple between carbon and hydrogen. Table 4 showed the experimental result of percentage of water-soluble extractives, hexane-soluble extractives, cyclohexane/ethanol-soluble extractives, and comparison with literature.

Table 4 - Chemical composition of *Eugenia paracatuana* and *E. francavilleana*.

	Experimental result (%)		**Comparison**			
	Eugenia paracatuana	*Eugenia francavilleana*	*Syzygium cumini*	*Eugenia involucrat a*	*Eucalyptus urophylla*	*Eucalyptus citriodora*
Water soluble extracts	11,09±1,56	25,04±1,86	-	-	-	4,16
Hexane-soluble extractives	15,28±2,71	24,89±2,19	-	22,50	-	8,54
Extracts soluble in cyclohexane / ethanol	1,39±0,18	0,20±1,18	2,37	-	0,31	-

Source: Authors.

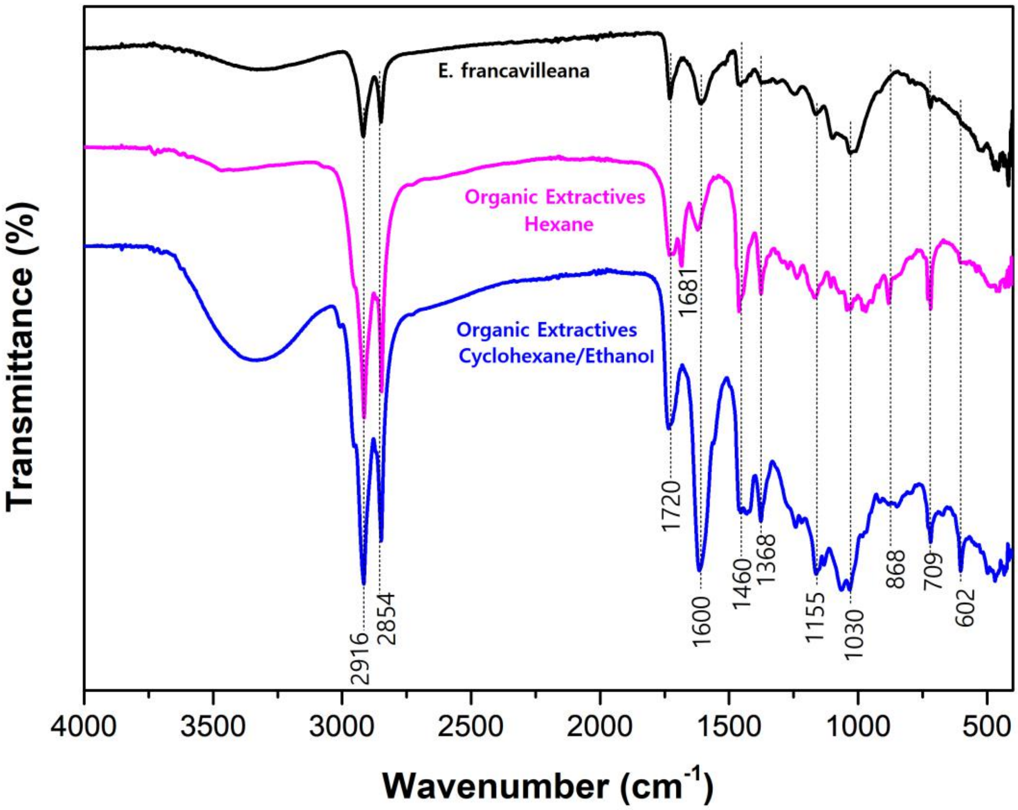
Percentage of water-soluble extractives was very high (11,09% and 25,04% for *Eugenia francavilleana* and *E. paracatuana*, respectively), indicating that material used was quite impure, impurities such as earth, sugar, and soot. In addition to possibly having more polar compounds in composition of extractives. Solvent polarity was the main parameter taken into consideration to discuss yield of extractives soluble in hexane and cyclohexane/ethanol. Presence of less polar compounds in plant tissue structures made hexane a better solvent than cyclohexane combined with ethanol. Oils were absolutely hydrophobic and therefore hexane was best solvent for them. Ethanol was polar and did not dissolve OrgExt (Liu et al., 2018; Souza et al., 2011). When comparing experimental result with results found in literature (Migliato, 2005), we could see that percentage of water-soluble extractives was actually quite high, indicating that material was impure or that there was a loss in process. And that extractives soluble in presented organic solvents, hexane and cyclohexane/ethanol, did not show a great difference compared to literature (Machado et al., 2014; Sato et al., 2018; Souza et al., 2011).

3.7 FTIR Analysis

Of thirteen oils extracted from species of *Eugenia* genus studied by Azevedo (Azevedo, 2014), 133 chemical constituents were characterized, being 26 monoterpenes and 107 sesquiterpenes. In most samples, predominance of oxygenated compounds was both mono- and sesquiterpenes. In addition to main compounds, it was important to know chemical structure of solvents so we could understand behavior of molecules.

FTIR analysis was performed on dried leaves "in natura" after removing soluble extractives in water and organic solvents. This analysis aimed to determine main functional groups presented in samples and verify whether pre-treatment changed chemical composition of leaves (Figure 3). SI 1 presented main components found in leaves of species *Eugenia francavilleana*. Characteristic bands of alkanes (1600 cm^{-1}), amines (1030 cm^{-1}), and alkyl halides (709 cm^{-1}) were found. This result was expected, since same bands were found in literature for Myrtaceae family, more specifically in *Syzygium cumini* (Sangeetha et al., 2014).

Figure 3 - FTIR spectrum of *Eugenia francavilleana*.



Source: Authors.

After analyzing spectrum represented in Figure 3 and SI 1, and comparing them with literature, it was concluded that after pre-treatment applied to "in natura" leaves, its main bands were maintained. However, the stretch at 3500 cm^{-1}, after extraction with the cyclohexane/ethanol solvent, was attributed to hydroxyl group (OH), this occurred due to association of OH of ethanol, which may be residual hydroxyls from solvent used in extraction. Both chemical structures, hexane (alkane) and cyclohexane (alkane and aromatic), had simple carbon bonds to hydrogen, so this increase noted in bands 1720 cm^{-1}, 2916 cm^{-1} and 2854 cm^{-1} was attributed to interactions between solvents. Same analysis was performed for leaves of species *E. paracatuana*, Figure 4. Main components found were presented in SI 2. It was possible to verify presence of amines (1050 cm^{-1}), alkanes (1674 cm^{-1}), aromatic (1457 cm^{-1}), carboxylic acids (960 cm^{-1}), and halides of alkyl (723 cm^{-1}).

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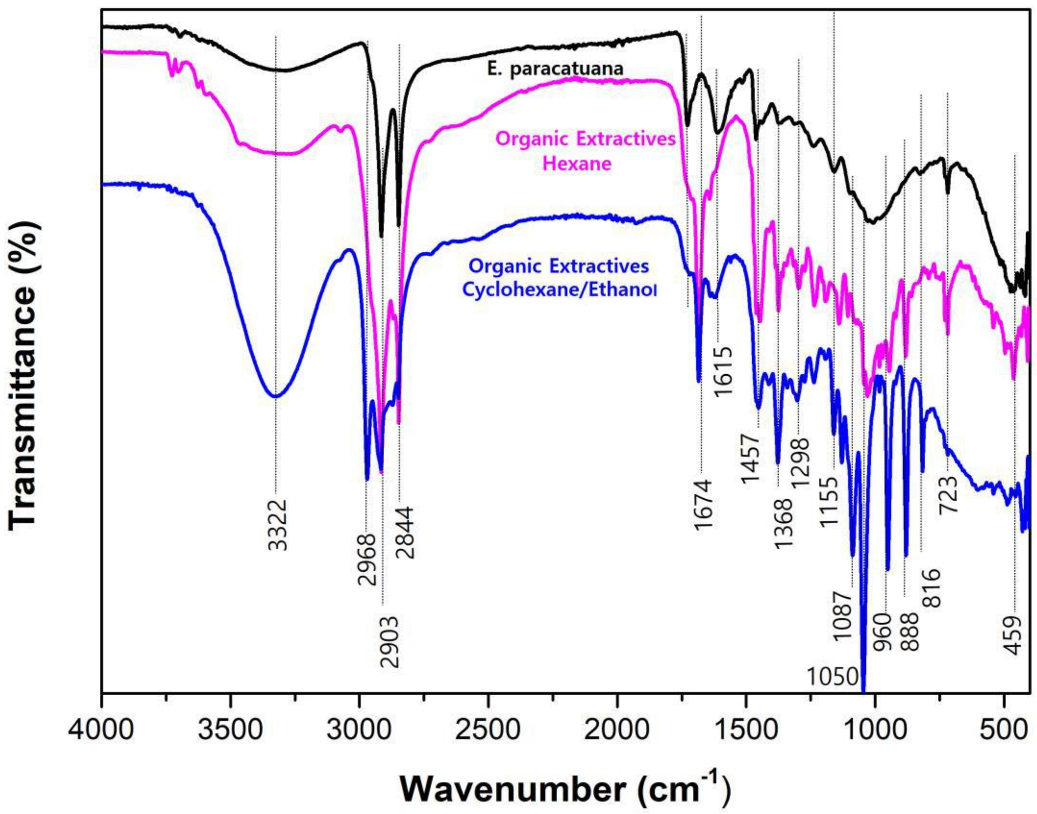
Figure 4 - FTIR spectrum of *Eugenia paracatuana.*



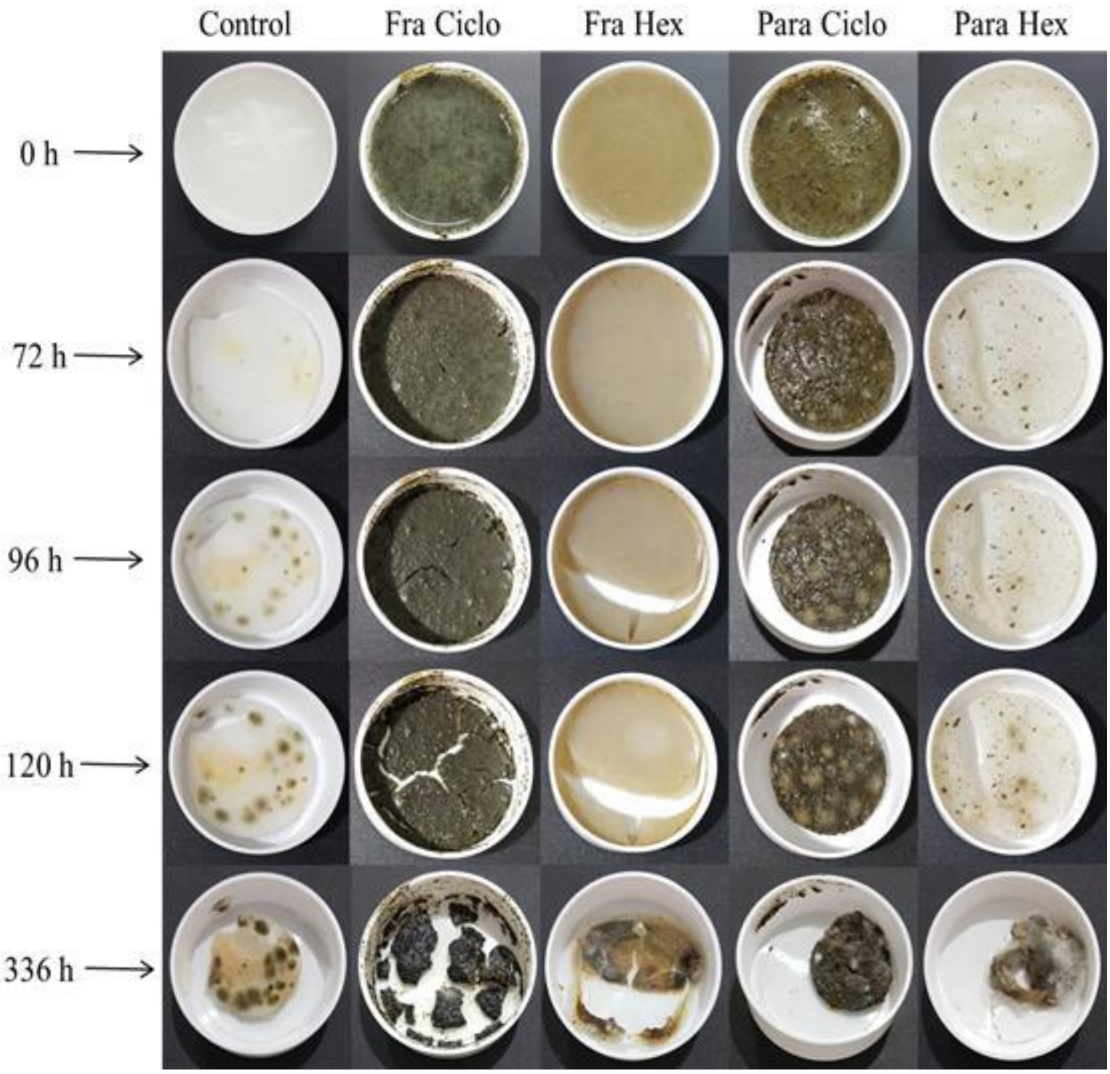
Source: Authors.

After comparing species studied in this work, same phenomenon of hydroxyl increase was observed in both graphs. Main bands took place in practically the same region. Spectra (Figure 3 and Figure 4) and Supporting Information (SI 1 and SI 2) containing components found in both species were compared with literature (Sangeetha et al., 2014), species used as a comparison was *Syzygium cumini* (presented as its synonymous *Eugenia jambolana*). About 40% of components found in spectrum of *E. francavilleana* were also found in *Syzygium cumini*. And about 35% of components found in spectrum of *E. paracatuana* were found in *Syzygium cumini,* components such as phenols, aromatics, alkanes, aliphatic amines, and alkyl halides. Analysis confirmed presence of phenol, which is known for its various properties, including being an effective antimicrobial (Monteiro, 2015).

3.8 Microbiological analysis

Films of commercial corn starch, OrgExt of *Eugenia francavilleana* and *E. paracatuana*, both extracted with solvent hexane and cyclohexane/ethanol (after previous extractions), and water were observed and photographed for a period of 14 days, Figure 5.

Figure 5. Control corn starch films, of both species, extracted with hexane and cyclohexane/ethanol solvent and arranged in lines according to time.



Source: Authors.

Based on visual analysis of films obtained in experiment, it was possible to observe gradual loss of water suffered by samples, which became increasingly drier and split into smaller pieces. Films must be handled with gloves and inhalation must be avoided, since microorganisms presented, even environmental, could be opportunistic and capable of manifesting their pathogenicity in specific individuals if proper care was not taken (Maciel et al., 2020).

Regarding presence of microorganisms, daily analysis of films was recorded through photographs. Analysis of photographic images revealed that in the control sample (without OrgExt), growth of microorganisms was gradual. For samples treated with OrgExt, there was a difference in appearance time of microorganisms, demonstrating that although all had microorganisms, some were slower than others. This has showed a greater capacity to delay this development, which means that they presented capacity at different levels antimicrobial.

In Para Ciclo's OrgExt-treated film, growth was apparently faster when compared to other films. Films treated with OrgExt from Fra Ciclo and Para Hex showed similar microbial growth after 96 h. On the other hand, in films treated with OrgExt from Fra Hex, microbial growth took longer to occur, being observed only after 120 h, showing better antimicrobial efficiency. According to Cardoso (Cardoso, 2021), phenolic substances were found in extraction of fruits of *Campomanesia* *xanthocarpa*, a species that also showed antimicrobial activity. So barrier to the apparent growth of microorganisms could be attributed to phenol, as described in literature (Monteiro, 2015), presented in the OrgExt of species added to films, corroborating results obtained in this work.

A probable explanation for why there has not been a total inhibition of growth of microorganisms by OrgExt is degradation of oils. This may have occurred due to exposure to light, oxidation of material due to contact with oxygen, and degradation due to presence of heat in extraction and preparation of films (Maciel et al., 2020).

However, although there was no complete inhibition of microbial growth in any of films produced, those prepared with *E. francavilleana* OrgExt extracted with hexane were able to significantly delay this activity. It is already common knowledge that several types of OrgExt had a broad spectrum of antibacterial, antifungal, and even antiviral activity. Since general mode of action varies in each case. In fungal pathogens, for example, OrgExt could cause damage to cell wall and disintegration of mitochondrial membrane, interfering with pathway of telectron transport system. While in bacterial pathogens, OrgExt usually acts in destabilizing cell architecture, degrading membrane integrity, disrupting many cellular activities, among others. OrgExt could also have antiviral activities against many RNA and DNA viruses (Tariq et al., 2019).

4. Conclusion

Based on results obtained and discussed in this study, it was possible to conclude that among organic solvents tested for extraction of essential substances, the most efficient was hexane, being *Eugenia francavilleana* the species that provided highest yield of extracted oil. As for ash content, on average, for both species it was higher than what we have found in literature for other *Eugenia* species. Humidity found for *E. paracatuana* was also lower than what we have found in literature. Moisture found for *E. francavilleana* is within values observed in literature. Results were consistent as they were compared to different species of same family and genus.

Compositional analysis by EDS revealed that main elements found in ashes of *E. francavilleana* were calcium, oxygen, and magnesium. And for ashes of *E. paracatuana* were calcium, oxygen, silicon, aluminum, potassium, and sulfur. Analyzes of the content of insoluble Klason Lignin revealed that *E. francavilleana* has the highest percentage of lignin content. As for the content of α-cellulose present, *E. paracatuana* presenting expected values based on literature. For *E. francavilleana,* value was lower than expected. For content of polyoses both classes of values were compatible with literature.

Regarding extractives, percentage of water-soluble extractives was very high. While extractives soluble in solvents provided hexane and cyclohexane/ethanol, they did not dissipate much difference compared to literature. Components found in both species by FTIR were compared with species *Syzygium cumini*. So, it was possible to verify similarity of composition with presence of phenols, aromatics, alkanes, aliphatic amines, and alkyl halides. Regarding antimicrobial analysis, both species presented late antimicrobial growth generation compared to control, and films treated with *E. francavilleana* oil extracted with hexane showed better antimicrobial efficiency.

In short, our results provide subsidies for future studies of OrgExt from leaves of *E. paracatuana* and *E. francavilleana,* aiming at the use of these OrgExt as plasticizing agents and investigation of their antimicrobial potential. As the work is pioneering, new solvents must be tested for the extraction of OrgExt in the species studied, as well as an in-depth study of their allo of inhibition.

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