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COMPARATIVE ANALYSIS OF EXTRACTION METHODS FOR RECOVERY OF SECONDARY METABOLITES FROM ESPINHEIRA-SANTA (*Maytenus ilicifolia*) USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Maytenus ilicifolia, a plant species native to Brazil, has a long history of use in traditional medicine for the treatment of various diseases. It has high pharmacological potential owing to the presence of secondary metabolites, such as phenolic compounds, which have been identified in leaf extracts by multiple studies. However, no study has yet investigated different methods for the extraction and identification of secondary metabolites from young plants. This study aimed to compare the efficiency of different extraction methods for spectral detection of secondary metabolites in young and adult specimens of *M. ilicifolia* using high-performance liquid chromatography/mass spectrometry. In the laboratory, *M. ilicifolia* seeds were sown in a germination tray and incubated in a germination chamber. After germination, seedlings were separated from the cotyledons and stored in an ultrafreezer. Leaf samples were collected from adult plants maintained under field conditions and immediately stored in an ultrafreezer. All samples were freeze-dried and subjected to different extraction methods (maceration, infusion, and ultrasonication) using ultrapure water or absolute ethanol as solvents. Extracts were then analyzed by HPLC/MS. Chromatographic analysis revealed that the aqueous extracts obtained by ultrasonication of seedlings and leaves and the aqueous extract obtained by infusion of cotyledons contained a greater number of compounds. Water extraction proved to be more efficient for all tested samples, precluding the need for organic solvents. In agreement with previous reports on leaf extracts, some compounds were only identified in *M. ilicifolia* seedlings. This finding underscores the importance of studying plants during all developmental stages to determine the metabolic fingerprint of the species and thus ensure adequate extraction of molecules of interest.

Keywords: Medicinal Plant. Infusion. Maceration. Ultrasound. HPLC.

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ANÁLISE COMPARATIVA DOS MÉTODOS DE EXTRAÇÃO PARA RECUPERAÇÃO DE METABÓLITOS SECUNDÁRIOS DA ESPINHEIRA-SANTA (*Maytenus ilicifolia*) UTILIZANDO CROMATOGRAFIA LÍQUIDA DE ALTA EFICIÊNCIA

RESUMO

A espinheira-santa (*Maytenus ilicifolia*) é uma espécie nativa do Brasil e vem sendo utilizada há décadas pela medicina popular no tratamento várias doenças, devido ao seu potencial farmacológico promovido pelos metabólitos secundários, como compostos fenólicos, terpenos e alcaloides, identificados em vários estudos realizados com extratos das folhas. Porém, ainda não existem estudos de métodos de extração de metabólitos secundários e identificação destes metabólitos na planta jovem. Assim, o presente trabalho teve por objetivo comparar a eficiência de diferentes métodos de extração para a detecção espectral de metabólitos secundários por cromatografia líquida de alta eficiência acoplada à espectrometria de massas (HPLC/MS), da planta jovem e adulta de espinheira santa. Em laboratório, as sementes foram dispostas em gerbox e levadas para câmara de germinação. Após germinadas, separou-se as plântulas dos cotilédones e armazenou-se em ultrafreezer a (-34 ºC). Amostras das folhas de plantas adultas foram coletadas em campo e imediatamente armazenada no ultrafreezer, também. Todas as amostras foram liofilizadas e submetidas à extração por diferentes métodos: maceração, infusão e ultrassom, utilizando como solventes água ultrapura e etanol absoluto e submetidas à análise cromatográfica. Os cromatogramas obtidos revelaram que os extratos aquosos de plântulas e folhas obtidos por sonicação, assim como extrato aquoso de cotilédones obtido por infusão, exibiram maior número de componentes. Assim, conclui-se que a extração com água foi mais eficiente para todas as amostras, evitando o uso de solventes orgânicos. A identificação de algumas moléculas na fase jovem de *M. ilicifolia*, como já encontradas por vários trabalhos nas folhas, mostra também a importância de estudar a planta desde o seu desenvolvimento inicial para obter um print metabólico e extração adequada para cada molécula de interesse.

Palavras-chave: Planta Medicinal. Infusão. Maceração. Ultrassom. HPLC.

ANÁLISIS COMPARATIVO DE LOS MÉTODOS DE EXTRACCIÓN PARA LA RECUPERACIÓN DE METABOLITOS SECUNDARIOS DE LA ESPINHEIRA-SANTA (*Maytenus ilicifolia*) UTILIZANDO CROMATOGRAFÍA LÍQUIDA DE ALTA EFICIENCIA

RESUMEN

Maytenus ilicifolia, una especie vegetal nativa de Brasil, tiene una larga historia de uso en la medicina tradicional para el tratamiento de diversas enfermedades. Posee un alto potencial farmacológico debido a la presencia de metabolitos secundarios, como los compuestos fenólicos, que han sido identificados en extractos de hojas por múltiples estudios. Sin embargo, hasta el momento, ningún estudio ha investigado diferentes métodos para la extracción e identificación de metabolitos secundarios en plantas jóvenes. Este estudio tuvo como objetivo comparar la eficiencia de diferentes métodos de extracción para la detección espectral de metabolitos secundarios en ejemplares jóvenes y adultos de *M. ilicifolia*, utilizando cromatografía líquida de alta eficiencia acoplada a espectrometría de masas (HPLC/MS). En el laboratorio, se sembraron semillas de *M. ilicifolia* en una bandeja de germinación e incubaron en una cámara de germinación. Después de la germinación, las plántulas se separaron de los cotiledones y se almacenaron en un ultracongelador. Las

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muestras de hojas se recolectaron de plantas adultas mantenidas en condiciones de campo y se almacenaron inmediatamente en un ultracongelador. Todas las muestras fueron liofilizadas y sometidas a diferentes métodos de extracción (maceración, infusión y ultrasonido) utilizando agua ultrapura o etanol absoluto como disolventes. Posteriormente, los extractos fueron analizados por HPLC/MS. El análisis cromatográfico reveló que los extractos acuosos obtenidos por ultrasonido de plántulas y hojas, así como el extracto acuoso obtenido por infusión de cotiledones, contenían un mayor número de compuestos. La extracción con agua demostró ser más eficiente para todas las muestras analizadas, eliminando la necesidad de disolventes orgánicos. En concordancia con estudios previos sobre extractos de hojas, algunos compuestos solo fueron identificados en plántulas de *M. ilicifolia*. Este hallazgo destaca la importancia de estudiar las plantas en todas las etapas de desarrollo para determinar la huella metabólica de la especie y, así, asegurar una extracción adecuada de las moléculas de interés.

Palabras clave: Planta Medicinal. Infusión. Maceración. Ultrasonido. HPLC.

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1 INTRODUCTION

Since the dawn of humanity, diseases have been one of the main problems faced by the population, regardless of ethnicity or social class. However, man began to look to nature for a cure for these illnesses and the use of medicinal plants proved to be efficient in their treatment, becoming a landmark for traditional medicine (Rodrigues et al., 2006; Morsli et al., 2021). Therefore, the study of these plants became necessary to find out how they act, which agents are responsible, their action, target and how they can be extracted.

According to Mahapatra et al. (2022), plants are sources of secondary metabolites and currently there has been increased attention to these plant-derived compounds, due to their effectiveness, ease of cultivation and handling. In addition, these elements are naturally synthesized by plants in response to biotic or abiotic factors, such as: temperature variation, water availability, nutrients present in the soil, ultraviolet radiation, attack by pathogens, fires, pollution, competition interference and allelopathy with other species, application of herbicides, among others (Gobbo-Neto and Lopes, 2007; Rockenback et al., 2018).

Medicinal plants have several natural compounds, which are responsible for the pharmacological activities of these plants. According to Taiz and Zeiger (2009); Barrios-Gonzales, (2018), secondary compounds differ from primary compounds because they are restricted to a species or groups of plant species and because they do not act directly in processes such as photosynthesis, protein synthesis, translocation, respiration and nutrient assimilation. In addition, it is worth noting that secondary products have different metabolic pathways and are therefore divided into three distinct chemical groups, such as: Terpenes, Alkaloids (nitrogenous compounds) and Phenolic Compounds (Ali, 2021).

Among medicinal plants, *Maytenus ilicifolia* Mart. Ex Reissek is a plant native to Brazil and is distributed in the southern region of the country and is easily found in native and riparian forests, where temperatures are mild and soils are rich in organic matter (Silva and Isaide, 2006; Oliveira et al. , 2009; Olivaro et al., 2021). This species belongs to the Celastraceae family and its leaves are serrated, leaving their ends pointed (Judd et al., 2009), and because of this characteristic and its pharmacological properties, this plant is popularly known as espinheira santa and has been used to popular medicine for decades in the treatment of pathologies such as gastritis, dyspepsia, gastric ulcer (Ecker et al.; 2017; Siqueira et al., 2019; Figueiredo et al., 2021), and recently it has been studied whether it has efficacy in diabetes treatment (Schindler et al., 2021).

In studies previously carried out by several authors, using leaves of adult plants,

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phenolic compounds were identified, responsible for protecting plants against herbivores and pathogens (Souza et al., 2019), terpenes (Mariot and Barbieri, 2007; Santos et al., 2021) , condensed tannins such as catechin, epicatechin and epigallocatechin (Rodrigues Sá et al., 2017), flavonoids, which are antioxidant compounds (Olivaro et al., 2021), alkaloids, triterpenes, which are antiulcerogenic and polyphenols (Souza et al.; 2005 ; Zhang et al.; 2020; Schindler et al., 2021). However, there are still no studies on methods for extracting secondary metabolites and identifying these metabolites in the young plant (Peralta et al. 2022).

Brazil is the country with the greatest biodiversity in the world and among this diversity, there are medicinal plants, which have been used for thousands of years by civilizations in the treatment of diseases, due to their pharmacological properties. In Brazilian phytotherapy, the indigenous people were one of the first to use these plants empirically, which after scientific research proved to be effective and safe to use.

Secondary metabolites extracted from plants are of high economic interest and are widely used in the food, cosmetics and mainly pharmaceutical industries (Machado and Paixão, 2021). According to the Research and Information System for Developing Countries (RIS) report, the global herbal market reached US$ 746.9 billion in 2022 (Singh et al., 2022). In addition, the state of Paraná is the largest producer of medicinal, aromatic and spice plants in Brazil, corresponding to 90% of national production. With an area of six thousand hectares, production is around 18.6 thousand tons of herbs, generating revenue of around BRL 88.5 million in 2021, according to data from the Secretariat of Agriculture and Supply of the State of Paraná (Available at: https://www.agricultura.pr.gov.br/Noticia/Estado-e-destaque-no-Pais-na-producao-de-plantas-medicinais).

Knowing the importance of natural compounds, knowing the extraction methods is crucial, since the quantity and quality of the extract will depend on the extraction method used (Rahim et al., 2022). According to Santos (2020), extraction are methods used to selectively and completely remove substances or active fraction from a plant material, using appropriate liquids, that is, the product is obtained through the passage of a solvent (H_2O or ethyl alcohol), by parts of the plant ground or not, to remove the natural compounds of this material. Among the conventional extraction methods are: steam dragging, percolation, decoction, infusion and maceration (Gori et al., 2021).

In view of these points addressed, the need for more in-depth scientific studies is evident, such as refined and precise methodologies in the identification of bioactive

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compounds present in plants.

Thus, the present work aimed to use different extraction methods to analyze them by high performance liquid chromatography of the young and adult plant of espinheira santa and subsequent identification in a mass spectrometer.

2 MATERIALS AND METHODS

2.1 PLANT SEEDLING EXPERIMENT

In the laboratory, the seeds of espinheira santa (*Maytenus ilicifolia*), collected on November 15, 2021, in the municipality of Bourbonia-PR latitude -24º11'30" S and longitude -52º 09'49" W, (Google Earth), were selected according to size and shape, and twenty-five seeds were placed in 11 x 11 x11 cm gerbox boxes, containing substrate with vermiculite and moistened with 40 mL of distilled water, each box. After sowing, the seeds were taken to the germination chamber, with a 12-hour light/dark photoperiod at a constant temperature of 27 °C (Alvarenga et al., 2020).

After 18 days, the germinated seedlings were removed from the germination chamber and gerbox boxes, undergoing a cleaning process in running water and later in distilled water to remove excess substrate. Then, the cotyledons and seedlings were separated, kept in the ultrafreezer (–34 ºC) for 3 days and subsequently lyophilized. Lyophilized samples were stored at –34°C until use.

2.2 PLANT ADULT EXPERIMENT

Samples of espinheira santa leaf parts were also collected on November 15, 2021, in the municipality of Bourbonia-PR latitude -24º11'30" S and longitude -52º 09'49" W, (Google Earth). At the Biochemistry laboratory – UEM/farm, Umuarama – PR, the samples were partially crushed and immediately taken to the ultrafreezer at a temperature of -34ºC, for 3 days and subsequently lyophilized. Lyophilized samples were stored at –34°C until use.

2.3 SAMPLE DRYING AND EXTRACTION EXPERIMENTS

The lyophilized samples were divided into parts to obtain extractions by infusion, maceration and ultrasound. For each extraction method used, a sample was separated for extraction in ultrapure water (m/v) and the same sample for extraction in absolute ethanol (99%), according to the flowchart below. For ultrasound, the methodology described by Sandhu, (2021) was used, where samples of seedlings, cotyledons and leaves were kept in

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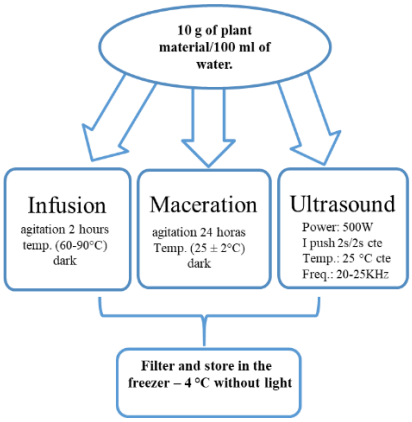
agitation by the probe for one hour with a maximum temperature of 40 ºC. In the infusion, the solvents were heated to around 60°C and each sample was stirred in the dark for two hours, with a temperature ranging from 60 to 70°C (Oliveira, 2016). In the maceration, the solvents were not heated and the samples were stirred for 24 hours at room temperature, in the dark (Cacique et al. 2020; Ferreira, 2020).

Flowchart 1

Flowchart of extraction methods: infusion, maceration and ultrasound



2.4 HPLC-DAD AND MS PREPARATION AND ANALYSIS

After all the extraction protocols performed, 1 mL of each sample was collected in eppendorfs, and these were centrifuged at 3,000 rpm. After centrifugation, the supernatant of the samples was collected and diluted in ultrapure methanol (80%). Soon after, these were filtered (PTFE L 0.45 µm syringe filter), and collected in vials to perform the analysis in HPLC/UHPLC-DAD (Shimadzu model NEXERA X2), coupled to the MS (Shimadzu model 8050) using the Method linear gradient for analysis of extracts (mobile phases milli Q water (A) and acetonitrile (B), PDA range 190-800nm, temperature maintained at 35°C, injection of 10 µl sample, C18 column (Shimadzu 5µm 150 x 4 .6 mm), total scan of 30 minutes, in the following schedule: 1-9 min (20% B), 10-15 min (40% B) and 16-30 min (10% B), (SOUZA, et al. , 2008) adapted.

Mass spectra were acquired in negative and positive ion mode. The instrument parameters were optimized before analysis and were: capillary temperature 300 °C, capillary voltage −57 V, spray voltage −2.8 KV and nebulizer gas flow (nitrogen) 3 L/min. In the ESI-MS n experiments, the most abundant ion or ion fragment was automatically selected as the precursor ion. Production ion spectra were recorded in the range of 100–1000 U m/z.

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The wavelengths selected for chromatogram analysis were 254 and 281 nm, whose values are within the range used by Quezadas (2022) for exploratory analysis of secondary metabolites in Pereskia aculeata Miller (Quezadas, 2022).

2.5 STATISTICAL ANALYSIS

The chromatograms and spectral masses were performed using the LabSolution LCMS program (post run, windows 7). The elaboration of tables and figures were carried out using Excel, GraphPad Prism® version 5 and Microsoft Power Point version 2013 programs.

3 RESULTS AND DISCUSSIONS

The efficiency of the extraction methods was compared based on (a) number of peaks, (b) relative amount of the major compound, inferred based on absorbance and (c) identification of previously known compounds by mass spectrometry (MS).

3.1 ESPINHEIRA-SANTA SEEDLING EXTRACTS

The aqueous extracts of espinheira-santa seedlings had a higher number of chromatographic peaks than ethanolic extracts, regardless of extraction method (infusion, 30 vs. 14; maceration, 27 vs. 16; ultrasonication, 39 vs. 25) (**Table 1**). In water extracts obtained by infusion and maceration, the major component (peak 5, retention time of 3.12 min, 34% of the total peak area, **Figure 1**) had an intensity close to 100 mAU, whereas the major component of the water extract obtained by ultrasonication had an absorbance of 210 mAU. By contrast, ethanolic extracts obtained by maceration, infusion, and ultrasonication had absorbance intensities of 20, 30, and 70 mAU, respectively (**Table 1**). It is noteworthy that some important metabolites were identified in the aqueous extract obtained by ultrasonication at a retention time of 3.0 min, including flavonoids such as catechin, epicatechin, and epigallocatechin (**Table 4**). These findings suggest that (i) water is more efficient than ethanol in extracting secondary metabolites from espinheira-santa and (ii) ultrasonication is more efficient than infusion and maceration.

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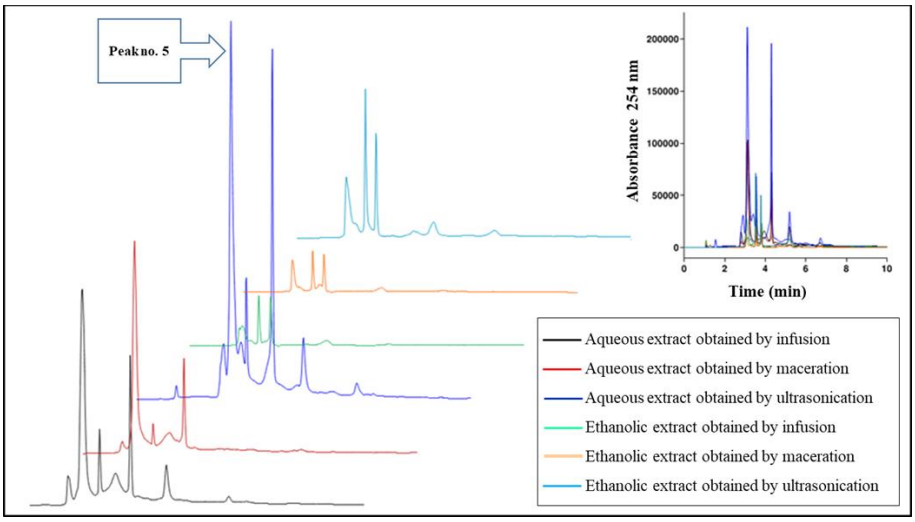
Table 1

Chromatographic data of Maytenus ilicifolia seedling extracts obtained using different solvents and extraction methods (Figure 1).

Extraction method	Aqueous extract		Ethanolic extract	
	Number of peaks	Maximum peak intensity	Number of peaks	Maximum peak intensity
Infusion	30	105 mAU	14	30 mAU
Maceration	27	100 mAU	16	20 mAU
Ultrasonication	**39**	**210 mAU**	25	70 mAU

Figure 1

HPLC-DAD chromatograms of Maytenus ilicifolia seedling extracts obtained using different solvents and extraction methods. The detailed plot shows superimposed chromatographic profiles. Absorbance readings were taken at 254 nm.



Ultrasonication has several applications in the food industry, being used for extraction, processing, preservation, emulsification, and homogenization (Chemat et al., 2011). Ultrasound-assisted extraction is a valuable method that preserves extractable compounds, minimizes the consumption of organic solvents, and reduces extraction time. In this technique, small sample volumes are treated in a bath that dissipates ultrasonic waves, typically in an aqueous medium (Vilkhu et al., 2008). The results of the current study showed that ultrasonication of young plant tissues (seedlings) using water as solvent was more efficient and sustainable than the other methods. Water extraction proved to be more efficient than ethanol extraction, likely because water molecules form hydrogen bonds with several classes of secondary metabolites, favoring the extraction of polar molecules. This result is

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advantageous, given that water is a highly accessible, easy to handle, and environmentally friendly solvent.

It is noteworthy that, at a wavelength of 190 nm, the major compound of aqueous extracts obtained by maceration, infusion, and ultrasonication had an absorbance intensity of 950, 1500, and 3750 mAU, respectively (data not shown). In this region of the light spectrum (far ultraviolet), other types of molecules can be identified, such as primary molecules associated with metabolism and cell structure (Santi et al., 2014).

3.2 ESPINHEIRA-SANTA COTYLEDON EXTRACTS

Analysis of cotyledon extracts revealed a higher number of peaks in aqueous extracts obtained by either infusion or maceration (**Table 2**). The absorbance intensity of the major compound of these extracts was highest in aqueous (350 mAU) and ethanolic (260 mAU) samples obtained by infusion (**Figure 2**). The highest-intensity peak of water extracts was observed at a retention time of 4.3 min. For samples extracted with ethanol, the highest absorption was achieved at a retention time of 3.5 min. Such differences were likely due to the type of solvent. For instance, ethanol interacts less intensely with the column than water.

Table 2

Chromatographic data of Maytenus ilicifolia cotyledon extracts obtained using different solvents and extraction methods (Figure 2).

Extraction method	Aqueous extract		Ethanolic extract	
	Number of peaks	Maximum peak intensity	Number of peaks	Maximum peak intensity
Infusion	**41**	**350 mAU**	24	260 mAU
Maceration	33	110 mAU	18	120 mAU
Ultrasonication	22	210 mAU	20	110 mAU

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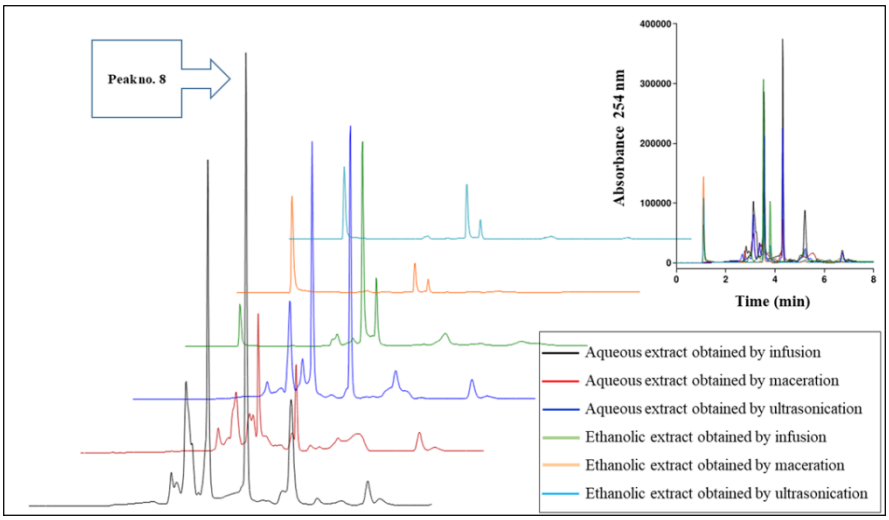
Figure 2

HPLC-DAD chromatograms of Maytenus ilicifolia cotyledon extracts obtained using different solvents and extraction methods. The detailed plot shows superimposed chromatographic profiles. Absorbance readings were taken at 254 nm.



The results demonstrated that aqueous infusion was the most efficient method for extraction of secondary metabolites from cotyledons (**Table 2**). This observation is important, as the cotyledon (i.e., shell-shaped concavity) produces the first leaves of an embryo. Its function is the storage of reserves for germination. This compact tissue has a physical barrier impairing molecule extraction. It is believed that the high temperature used here (90 °C) contributed to breaking the barrier, resulting in infusion being the most efficient method for cotyledon samples.

In the absorption spectrum of the water extract obtained by infusion, the largest peak was peak 8, with maximum absorption at 254 nm, retention time of 4.3 min, and relative area of 28% (**Figure 2**). These findings agree with the results of HPLC/MS. Catechin, epigallocatechin, epigallocatechin gallate, quinic acid, syringic acid, linolenic acid, and phloretin xylosyl-galactoside were identified at a retention time of about 4.0 min (**Table 4**).

3.3 ESPINHEIRA-SANTA LEAF EXTRACTS

Aqueous leaf extracts had a higher number of components than ethanolic extracts, regardless of extraction method (infusion, 59 vs. 45; maceration, 56 vs. 44; and ultrasonication, 59 vs. 49) (**Table 3**). The absorbance intensity of the major compound was higher in samples obtained by ultrasonication (**Figure 3**), with the highest absorbance intensity (500 mAU) observed in the aqueous extract. Therefore, as was found for seedling

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extracts, ultrasound-assisted aqueous extraction was the most efficient method for obtaining leaf extracts.

The spectrum of the aqueous leaf extract obtained by ultrasonication (**Figure 3**) shows that, up to 9 min of extraction, peak 6 had the highest intensity (450 mAU) at 254 nm and a retention time of 4.3 min. From 10 to 15 min, peak 35 exhibited the highest intensity (500 mAU) at a retention time of 13.7 min. For the ethanolic extract obtained by ultrasonication, peak 27 had the highest intensity (200 mAU), albeit lower than that of peak 8 of the aqueous extract, at the same retention time. Overall, it can be said that aqueous extraction was more effective, even for less polar molecules.

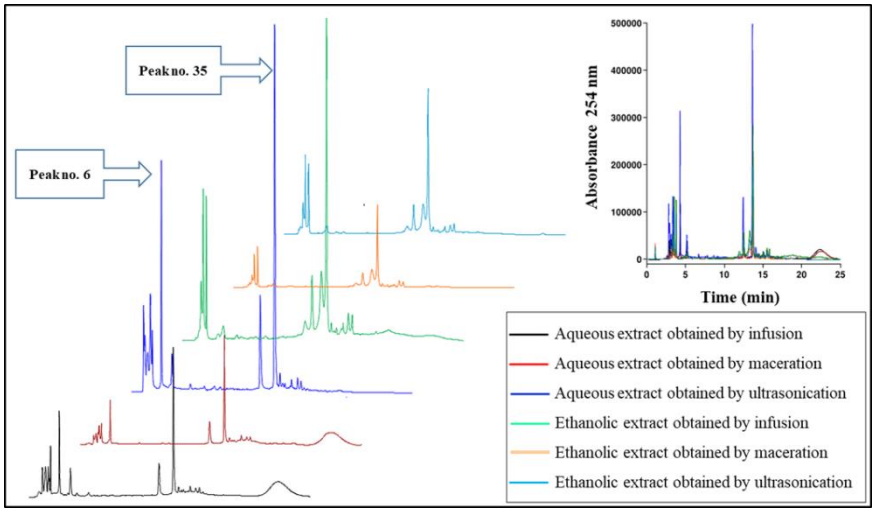
Table 3

Chromatographic data of Maytenus ilicifolia leaf extracts obtained using different solvents and extraction methods (Figure 3).

Extraction method	Aqueous extract		Ethanolic extract	
	Number of peaks	Maximum peak intensity	Number of peaks	Maximum peak intensity
Infusion	59	200 mAU	45	280 mAU
Maceration	56	150 mAU	44	110 mAU
Ultrasonication	**59**	**500 mAU**	49	200 mAU

Figure 3

HPLC-DAD chromatograms of Maytenus ilicifolia leaf extracts obtained using different solvents and extraction methods. The detailed plot shows superimposed chromatographic profiles. Absorbance readings were taken at 254 nm.



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The absorbance spectrum of the aqueous leaf extract obtained by ultrasonication revealed peaks 6 (retention time of 4.3 min, 32% of the total area) and 35 (retention time of 13.7 min, 32% of the total area) to be the largest, as shown in **Figure 3**. In the same sample (**Table 4**), HPLC/MS identified catechin, epicatechin, epigallocatechin, quinic acid, syringic acid, and linolenic acid at a retention time of 4.0 min. At a retention time of 13 min, the identified compounds were phloretin xylosyl-galactoside, rutin, brassicasterol acetate, and kaempferol-di-(rhamno)-hexoside.

3.4 COMPARISON OF SECONDARY METABOLITES AT DIFFERENT DEVELOPMENTAL STAGES

The mass spectra of precursor ions of molecules detected by HPLC/MS were analyzed according to plant developmental stage. The analysis was performed with aqueous extracts only, as this solvent was the most effective. **Table 4** presents the major metabolites detected, including flavonoids such as catechin, epicatechin, epigallocatechin, and epigallocatechin gallate. The results are in agreement with previous studies on the leaf extract of espinheira-santa (Souza et al., 2008; Zhang et al., 2020; Ali et al., 2021).

Identification and comparison of secondary metabolites according to plant development (seedling, cotyledon, and leaf) are very important, as, according to Antunes (2019), phenolic compound accumulation is greatly influenced by nutritional stress, pathogens, UV radiation, temperature, and exposure to herbicides. Therefore, identification of metabolites generated at the beginning of plant development facilitates the identification of molecules activated by genes that are expressed throughout life, even under adverse conditions.

As listed in **Table 4**, catechin was detected in the aqueous extracts of all parts of espinheira-santa (seedling, cotyledon, and leaf) at a retention time of 3.0 min, regardless of extraction method. Epicatechin was identified in all extracts, except seedling extracts obtained by infusion and maceration. Epigallocatechin and epigallocatechin gallate were observed mainly in seedling and leaf extracts obtained by ultrasonication. These three compounds were identified in cotyledon extracts obtained by infusion.

Other classes of molecules were identified, including the phenolic compounds quinic acid, syringic acid, rutin, and shikimic acid. Rutin was detected only in the leaf extract obtained by ultrasonication. Quinic acid was identified in all extracts, with the exception of the seedling extract obtained by maceration. Some molecules of the terpene class were also

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detected, such as brassicasterol acetate and kaempferol. The former was identified only in leaf extracts, particularly those obtained by ultrasonication, as was kaempferol. These results corroborate those of Tiberti et al. (2006), who identified brassicasterol acetate and kaempferol in espinheira-santa leaves.

Table 4

Precursor ions and retention times (t_R) of aqueous extracts obtained by infusion, maceration, and ultrasonication of Maytenus ilicifolia seedlings (S), cotyledons (C), and leaves (L), as determined by high-performance liquid chromatography/mass spectrometry in negative and positive ion modes.

Compound	[M–H] (*m/z*)	Infusion, t_R (min)			Maceration, t_R (min)			Ultrasonication, t_R (min)		
		S	C	L	S	C	L	S	C	L
Catechin ($C_{15}H_{14}O_6$)	+291	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Epicatechin ($C_{15}H_{14}O_6$)	−289	-	2.0	2.0–11.0	-	-	7.0–11.0	2.0–7.0	2.0	2.0–11.0
Epigallocatechin ($C_{15}H_{14}O_7$)	−305	3.0–7.0	2.8–5.0	2.0–6.0	3.0	-	2.0–12.0	3.0	2.5–6.0	2.0–10.0
Epigallocatechin gallate ($C_{22}H_{18}O_{11}$)	+459	-	4.0–7.0	3.0	17.0	-	-	16.0	-	9.4
Quinic acid ($C_7H_{12}O_6$)	−191	3.0–15.0	2.0–5.0	3.0–4.0	-	2.0–12.0	3.0–14.0	2.0–12.0	2.0–6.0	2.0–8.0
Syringic acid ($C_9H_{10}O_5$)	+199	-	3.0	3.0	2.0–3.0	-	-	-	3.0–5.0	3.0
Linolenic acid ($C_{18}H_{32}O_2$)	−279	4.0–15.0	5.0–18.0	4.0–17.0	17.0–27.0	-	4.0–20.0	4.0–19.0	-	4.0–21.0
Phloretin xylosyl-galactoside ($C_{26}H_{32}O_{14}$)	+569 −567	-	4.0	14.0–28.0	-	-	4.0–15.0	-	-	11.0–22.0
Rutin ($C_{27}H_{30}O_{16}$)	−609	-	-	-	-	-	-	-	-	14.0
Brassicasterol acetate ($C_{30}H_{48}O_2$)	−439	-	-	-	-	-	8.0–10.0	-	-	7.0–10.0
Kaempferol-di-(rhamno)-hexoside	+741 −739	-	-	-	-	-	-	-	-	13.5
Kaempferol-rhamno-hexoside	+593	-	-	-	17.0–21.0	-	-	-	-	16.0–21.0
Shikimic acid ($C_7H_{10}O_5$)	+175 −173	-	-	-	-	4.0–20.0	-	-	4.0–15.0	-

4 CONCLUSION

Based on the experiments carried out and the results obtained, it can be concluded that, for the espinheira santa plant (*Maytenus ilicifolia* Mart. Ex Reissek), the extraction method using ultrasound proved to be more effective compared to the methods of infusion and maceration, because in addition to extracting a greater amount of components, the major compounds also showed greater intensities, both for seedlings and for the leaves of this plant. However, the most effective extraction method for the cotyledon was infusion, probably due to the temperature used and the rigid nature of the sample.

In all techniques used, water was the solvent with the highest extractive capacity, which allows for a cleaner extraction without the addition of organic solvents, providing greater sustainability in the extraction.

Therefore, the identification of molecules in the young phase of *M. ilicifolia*, as already found by several works in the leaves, also shows the importance of studying the plant from

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its initial development to obtain a metabolic print and adequate extraction for each molecule of interest.

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