



Characterization of Compounds in the Plant *Maytenus ilicifolia* Mart. Ex Reissek during the Initial and Adult Development Stage

**Wolfran Aparecido de Alvarenga¹, Érica Marusa Pergo Coelho^{1*}
Otávio Akira Sakai², Filipe Andrich³ and Camilla Yara Langer Ogawa⁴**

¹*Department of Agronomic Sciences, State University of Maringá - UEM, Umuarama Campus, Umuarama, Paraná, Brazil.*

²*Department of Physics, Federal Institute of Paraná – IFPR, Umuarama, Paraná, Brazil.*

³*Department of Biology, Federal Institute of Paraná – IFPR, Umuarama, Paraná, Brazil.*

⁴*Department of Physics, State University of Maringá – UEM, Maringá, Paraná, Brazil.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors ÉMPC and WAA conceptualized the idea. Authors ÉMPC, WAA, OAS and CYLO have done the methodology. Author OAS has done the software analysis. Author ÉMPC has done the validation of the work. Authors WAA and FA completed the formal analysis. Author OAS completed the investigation. Author WAA managed the resources. Author ÉMPC done the data curation. Author WAA done the writing and original draft preparation. Authors ÉMPC and WAA performed the writing-review and editing. Authors ÉMPC, WAA, OAS and FA completed the visualization. Authors ÉMPC and WAA have done the overall supervision. Author ÉMPC managed the project administration. Authors WAA and ÉMPC managed the funding acquisition. All authors read and approved the final manuscript.

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ABSTRACT

Maytenus ilicifolia Mart. Ex Reissek, was first studied in the 60s, but only in the 80s and 90s were the studies were driven by scientific research that attributed medicinal properties to the plant, such as healing actions for human gastric diseases. In this work, a study was carried out on the characterization of the chemical composition of *M. ilicifolia* at different stages of development, using

*Corresponding author: E-mail: profericapergo@gmail.com;

spectroscopic techniques of sustainable character, which do not require toxic solvents as previous preparations. Extracts from cotyledons, seedlings that have never been researched in the literature and leaf/stem were lyophilized to characterize the compounds present in this plant using photoacoustic and spectroscopy techniques in the Infrared spectroscopy using Fourier Transform-FTIR with ATR. The photoacoustic results show that there is a differentiation of development stages in the plant due to the greater presence of chlorophylls and terpenes in the seedlings and their absence in the cotyledon, which is characterized as the reserve part in the germination phase of the plant. The range of bands the literature reports for epigallocatechin and friedelin, the possible agents responsible for the medicinal effects of the plant were mainly seen in the cotyledon, thus demonstrating the use of the seedling in its initial stage, to extract and obtain these active components.

Keywords: Spectroscopy; triterpenes; flavonoids; photoacoustic; FTIR-ATR.

1. INTRODUCTION

Maytenus ilicifolia Mart. Ex Reissek, popularly known as “espinheira-santa”, has this name because its leaves have thorns. This plant belongs to the family Celastraceae, is native to several regions of South America, present in the southern region of Brazil, with its predominance in mixed or dense ombrophilous forest in areas of higher altitude, and also occurs in seasonal semi-deciduous forests [1-4]. *M. ilicifolia* was classified as a species that prefers humid soils and riparian environments where it normally occupies and develops in rock outcrops in small areas and riparian forest environments [5]. According to Caldas and Matos [6], *M. ilicifolia* is a native plant from Brazil, with geographical distribution in Rio Grande do Sul, Santa Catarina, Paraná, São Paulo, Mato Grosso and Mato Grosso do Sul. It has a shrub habit of up to 3 meters height, with alternating, persistent, simple leaves, with a glabrous, leathery and spiny toothed margin.

As it is a plant widely used by the community to treat gastric diseases, *M. ilicifolia* is, for the most part, removed from nature in an extractive manner. Thus, some producers in Brazil cultivate the plant for producing herbal medicines, contributing to conserving the species in its natural environment. In Brazil, extracts of *M. ilicifolia* Mart., one of the 132 Celastraceous species native to the country, are used widely as phytotherapeutic drugs and are even made available through the publicly funded Brazilian health care system [7].

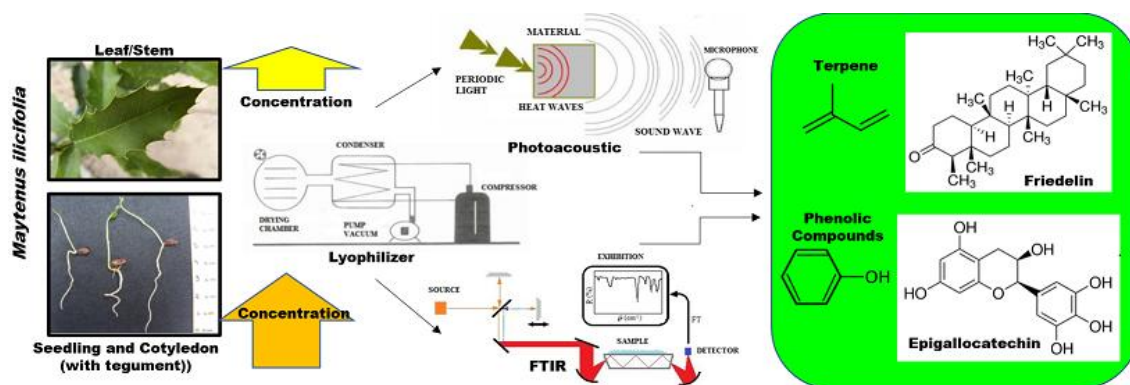
The first studies carried out with plants of the genus *Maytenus* spp. were carried out in the '60s by Lima, Coelho, Weigert, D'Albuquerque and Souza [8] the first researchers to dedicate themselves to studies of the phytochemical groups found in the leaves. In the 70s, it was

evidenced in studies carried out on the leaves, that *M. ilicifolia* had triterpenoid compounds in its composition, which were verified to have cytotoxic action against tumour cells in vitro research [9]. During the 1980s, studies were stimulated by their effectiveness in treating gastric ulcers, gastritis, dyspepsia and indigestion, properties that were later confirmed in the late 1980s [10] and more recently [11].

Among the various substances isolated and identified from the roots, leaves and branches in the studies carried out with *M. ilicifolia*, the ones that stand out the most are terpenes (isotenginone II and trigenone), triterpenes (maytenoic acid, friedenalol, friedelin and maytene), dimeric triterpenes (congorosins A and B, friedenelol), tannins (among them the gallic ones, epigallocatechin gallate, epicatechin and epigallocatechin, are the ones that stand out the most), glycolipids (monogalactosildiacilglicerol, digalactosildiacilglicerol and tetragalyglicerols mayine) and maytansine, [7, 10, 12-16].

Studies have shown that the chemical compounds responsible for treating gastric diseases in humans, especially tannins, which are derived from phenolic compounds, in particular epigallocatechin, which are major secondary compounds present in the leaves of *M. ilicifolia* [16,17], triterpenes, especially friedelin [14-16,18-19], and flavonoids, which are antioxidant compounds, [16,20].

Thus, it is extremely important to carry out further studies on chemical characterization and the corresponding identification techniques. In this work, a differential analysis was performed on the presence of bioactive compounds present in lyophilized extracts of *M. ilicifolia*. The leaf and stem are the most addressed part in studies found in the literature; however, there are no studies on compounds derived from secondary



metabolism present in lyophilized extracts of cotyledons and seedlings. Based on this point, the possible use of cotyledons and seedlings to obtain active compounds can be an alternative to the extractivism suffered by the species, positively impacting the maintenance of the plant in its natural environment. The feasibility of producing herbal products from cotyledons and lyophilized seedlings is a new alternative for use by the population. In the analysis and evaluation of compounds present in the plant, techniques were used that can be used to characterize molecules that significantly demonstrate the quality of the product, to avoid adulterations and contamination in samples of plant origin that were used in the research.

Thus, the objective of this work was to identify the compounds present in cotyledons and seedlings in the early stage of development and in the leaf/stem of the adult stage of the *Maytenus ilicifolia* plant using photoacoustic and ultraviolet (PAS)-visible and infrared spectroscopy (FTIR-ATR).

2. MATERIALS AND METHODS

2.1 General Experimental Procedure

The photoacoustic spectrum - PAS used an apparatus assembled by the Study Group of Photothermal Phenomena (GEFF). The apparatus has a Xenon arc light source (Oriol, 68820) with power set to 800 W. The light emitted by the lamp passes through a monochromator (1/8 m), (Oriol, 77250) with a diffraction grid for the region between 200 to 800 nm (UV-vis) and 3.16 mm slits at the entrance and exit of the monochromator. A mechanical modulator (Stanford Research Systems, SR 540), set at 25 Hz, controls the frequency of light modulation by providing a reference signal for

the amplifier (lock-in). FTIR - AGILENT CARY 630 FTIR SPECTROMETER, the interferometer system is of the Michelson type and has a 25 mm optical path, permanently aligned, with a mechanical flexural bearing. The interface used for the sample was a diamond ATR. The spectral range 4000 to 400 cm^{-1} was determined by the KBr disk with spectral resolution $<2 \text{ cm}^{-1}$, wave number accuracy 0.05 cm^{-1} and reproducibility of wave number 0.005 cm^{-1} . The equipment's power supply is 110–240 V AC, 60/50 Hz, The spectrometer uses the Agilent MicroLab PC Software, automated IQ/OQ, in compliance with 21 CFR.

2.2 Plant Materials and Extraction

The plant studied was *Maytenus ilicifolia* Mart. Ex Reissek. Adult leaves and stem were used, as well as cotyledons and seedlings from the initial development phase.

The leaf and stem of *M. ilicifolia*, grown in the municipality of Umuarama, Paraná, were obtained in partnership with Horto da Universidade Paranaense - UNIPAR and collected on March 26, 2018. A sample is filed at the Herbarium of Horto Medicinal de Campus 2 of UNIPAR under number 36. The remaining samples were subsequently crushed and frozen immediately, for approximately 7 hours in the ultra-freezer at -34°C . Afterwards, they were lyophilized (Lyophilizer LS 3000B), to remove all the water and then dry macerated in the grail.

The seeds used to obtain the seedlings were purchased in the city of Campo Largo, Paraná, from the company Chamel Indústria e Comércio de Produtos Naturais Ltda. In the laboratory, seeds were selected for size and shape, and twenty seeds were placed in an 11 x 11 x 3 cm gerbox, containing medium-sized vermiculite and moistened with 10 ml distilled water per each

plate. After sowing, the seeds were taken to a germination chamber, with a 12h light and 12h dark cycle at a constant 27°C. After 21 days of incubation, the cotyledons (without the integuments) that remained from the seedlings and seedlings with a maximum height of 8 cm, were dry macerated in the grail and immediately frozen for approximately 7 hours in the ultra-freezer at -34°C, followed by lyophilization in the Lyophilizer LS 3000B.

The lyophilized extracts, both from the leaf/stem of the adult phase, the cotyledons and the seedlings from the initial stage of development, were later used in spectroscopic analyses.

2.3 Photoacoustic Spectroscopy (PAS)

Photoacoustic spectroscopy (PAS) [21,22,23] studies the interaction of matter with incident radiation, modulated through the photoacoustic effect. The photoacoustic technique is characterized by obtaining photoacoustic spectra, which is proportional to the sample's optical absorption coefficient, depending on the modulation frequency. The main applications of this technique are largely for obtaining photoacoustic spectra of various types of materials, whether solid, semi-solid, gases or liquids, not requiring rigorous sample preparation for testing.

The photoacoustic spectroscopy analyses were carried out in the Photothermal Phenomena Study Group (GEFF) of the Physics Department of UEM. The conditions of the experiment for all 5 mg samples analysed were: Frequency: 25 Hz; Sensitivity: 100 mV; Time: 300 ms; Lamp Power: 800W; Averages per point: 10.

2.4 Infrared Spectroscopy by Fourier Transform- FTIR with ATR

Infrared spectroscopy using Fourier Transform-FTIR with ATR has the objective of obtaining the spectra of a large number of organic compounds in liquid, solid and vapour state. This infrared absorption occurs when the incident radiation frequency is in resonance with the vibration frequency of the molecule; however, there needs to be variation in its electric dipole moment [24,25,26]. The great advantage of using the ATR is that the solid sample/powder will not need preparation and the absorption or transmission spectra are reproducible. Another great advantage is that it requires a small amount of sample and can be used for both inorganic and organic compounds.

The spectroscopy measurements in the infrared region were carried out at the Complex of Support Centres for Research (COMCAP), which is linked to the Dean of Research and Graduate Studies/ Research Directorate/ Division of Research Support Centres from the State University of Maringá.

Lyophilized extracts of cotyledons, seedlings and leaf/stem were prepared in a potassium bromide (KBr) tablet. The spectra of the samples were analysed in an FTIR spectrophotometer with Bruker Vetex ATR, in the spectral range from 4000 to 400 cm^{-1} , with a resolution of 4 cm^{-1} and 128 scans for each 5 mg sample analysed.

3. RESULTS AND DISCUSSION

The photoacoustic spectroscopy analyses of the samples of *Maytenus ilicifolia*, with signal normalized to charcoal, from extracts of the leaf/stem, seedlings and cotyledons, can also be observed in (Tables 1, 2 and 3), respectively, showing that bands in the region of visible light that corresponds to electromagnetic radiation between 400 and 700 nm occurred only in the samples of the extracts leaf/stem and seedlings. In the cotyledon extract, the band area only appeared at 233.8 and 358.3 nm, which are lengths in the ultraviolet (UV) range.

In the leaf/stem extract, two areas of larger bands were observed between these wavelengths, the first area was 813.8 u.a at 499.9 nm and the second area was 718.0 u.a at 674.0 nm. In these two wavelengths, the band areas were identical to the data observed and shown in (Fig. 1) because, in this extract, the numbers of molecules that are proportional to the size of the area and the amount of energy are equal. 499.9 nm is close to blue, where there is absorption of light from various molecules involved in the photosynthesis process, such as chlorophylls and carotenoids (β -carotene), which are pigments that absorb light at wavelengths not absorbed by chlorophyll, thus being additional light receptors. 674.0 nm corresponds to red, which indicates the maximum light absorption spectrum of Photosystem II (P680) photosynthesis.

In photosynthesis, light (light energy) is transformed into chemical energy and can be dissipated in three ways, the photochemical pathway, the chlorophyll fluorescence pathway and the heat pathway. In fluorescence

Table 1. Photoacoustic spectroscopy analysis of the *Maytenus ilicifolia* leaf/stem sample

Wavelength (nm)	Spectral band number	Band area (ua)	Band width (ua)
254.0±6.9	1	20877.2±128.2	359.8±25.7
418.4±8.3	2	31.100±5.500	76.70±9.30
499.9±4.1	3	813.80±40.50	151.6±12.0
674.0±3.2	4	718.00±24.20	40.20±3.60

Residual Error = 0.992044

Table 2. Photoacoustic spectroscopy analysis of the *Maytenus ilicifolia* seedlings sample.

Wavelength (nm)	Spectral band number	Band Area (ua)	Band Width (ua)
245.0±3.70	1	7751.8±626.6	157.7±10.2
450.9±9.60	2	3371.9±170.2	157.5±9.80
626.3±11.9	3	785.50±31.70	76.1±21.50
679.1±2.30	4	464.10±22.50	39.1±7.000

Residual Error = 0.96153

Table 3. Photoacoustic spectroscopy analysis of the *Maytenus ilicifolia* cotyledons sample

Wavelength (nm)	Spectral band number	Band Area (ua)	Band Width (ua)
233.8±2.3	1	2527.1±341.4	110.4±6.74
358.3±2.0	2	3411.5±496.0	318.8±28.0

Residual Error = 0.95124

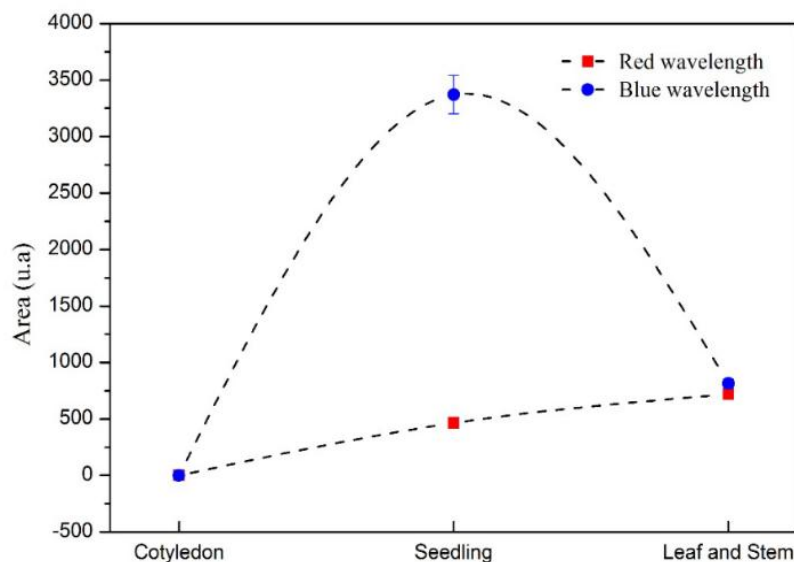


Fig. 1. Samples of leaf/stem, seedlings and cotyledon versus area (u.a), obtained from the photoacoustic spectroscopy analysis of *Maytenus ilicifolia*. The dashed line is a guide for the eyes

(Source: Author)

dissipation, photons are re-emitted over a longer wavelength with less energy, in a process called chlorophyll fluorescence [27].

Chloroplasts always contain both chlorophyll A and B. Although both are green, their absorption spectra are sufficiently different to

complement each other's range of light absorption in the visible region. Most plants contain twice as much chlorophyll A as B. These photosynthetic pigments have their peak photon absorption in the 428 and 660 nm and 452 and 641.8 nm regions (chlorophyll A and chlorophyll B, respectively) [28].

In the seedling extract, (Table 2), there are also two larger band areas in the visible light region similar to the wavelengths found in the leaf/stem extract. However, at 450.9 nm the size of the band area is 3,371.9 u.a, which is approximately 4 times larger than the band area at 626.3 nm (785.5 u.a) (Fig. 1). So, in this wavelength of visible light close to blue, the number of molecules, mainly the carotenoids (which are isoprenes), was increased in this extract.

These differences between the areas corresponding to the number of molecules found in these three extracts, identified by photoacoustic spectroscopy, allow the interpretation that even though these tissues are from the same species of plant, they have different amounts of molecules expressed by the gene of this species because they are in different stages of development. The cotyledon, for example, is a reserve tissue; therefore, they do not perform photosynthesis, but store molecules that are used in energy metabolism to facilitate the seed germination process. The seedling, which corresponds to the first stage of development after germination may present molecules involved in photosynthesis as well as molecules involved in cell elongation, as the energy metabolism for this development phase no longer depends on reserves but on the beginning of the photosynthetic process.

In the initial phase, the energy reserve tissue (cotyledon) did not show detectable chlorophyll in the analysed spectrum. This is an important fact since all the molecules present in this tissue do not depend on the photosynthetic process (chlorophylls) to produce other molecules. In this tissue, the molecules already existing in the seed are rearranged by enzymatic metabolism, for example, the glyoxylate cycle, to produce new molecules, with the sole purpose of propagating the species through the germination process.

Chlorophyll was found in the seedling, corresponding to the tissue originating from cotyledon germination, and in the adult phase of the plant (leaves); in these samples, it was possible to identify the bands in which the chlorophylls are active according to variations in wavelengths.

Based on the photoacoustic spectroscopy analyses, spectroscopy analyses were then performed in the infrared region to complement the data. (Fig. 2) shows the results for the

extracts of the cotyledon, seedling, and leaf/stem of the *Maytenus ilicifolia* plant. The graph is represented by wavenumber (cm^{-1}) versus intensity (a.u.) for better understanding.

The number of absorption bands is different for the three samples, 21 peaks, 11 peaks and 10 peaks for the cotyledon, seedling and leaf/stem, respectively. The peaks found in the 3600–2700 cm^{-1} region are generally associated with axial deformation vibrations in hydrogen atoms linked to carbon, oxygen and nitrogen (C-H, O-H and N-H). Absorption in the 1500–600 cm^{-1} region is associated with several types of vibration, including axial and angular deformations of carbon bonds with oxygen and nitrogen atoms, bonds between carbon and also with a radical group (CO, CN, CC and CX). (Table 4) correlates the absorption bands obtained for the studied samples and functional groups described in the literature [29,30].

The functional group assignments can show the possible existence and contribution of water (H_2O), protein, lipids, carbohydrates and cellulose. The cotyledon spectrum has a greater number of bands; however, the bands at 1517, 1242 and 1044 cm^{-1} did not appear in the same. The bands identified in this Table presuppose the presence of some molecules already identified in previous studies, among them the terpenes that oscillate in the 3290 cm^{-1} to 1740 cm^{-1} range. This range can be directly linked to the presence of fat-soluble compounds when compared to the study carried out by Ramos et al. [29], where they obtained data close to those in the analysis shown.

The band at 3290 cm^{-1} may be associated with olefin but it may also be the presence of free water, which could be associated with moisture present in the sample on the day of the experiment or even in the environment in which the analysis was performed. It is important to mention that olefin is not a compound described in the literature as present in *M. ilicifolia*. Thus, it can be assumed that the band found in the 3290 cm^{-1} range is associated with the presence of some compound correlated to the terpenes since they are compounds that have similar molecules. The basic unit of terpenes, which are isoprenes, are formed through the carbon captured in the primary metabolism.

It can be assumed that the 3290 cm^{-1} band is associated with the existing O-H bond in the same way in polyphenols (which are tannins) and

catechins. The existence of this band is also associated with the N-H groups [31]. The cotyledon and seedling samples have more prominent peak intensities and widths than the leaf/stem.

The 2922 and 2850 cm^{-1} bands are due to the C-H stretches of the CH₃ and CH groups, respectively. They can be present in organic compounds such as lipids and epigallocatechin [32].

According to Giglio et al. [21] mathematical treatments, such as peak area calculation by spectral integration or decomposition, are widely used in the quantification of components when it comes to infrared spectra. The peak area was calculated using the integral calculation in the Origin8.5 program, which is shown in (Fig. 3). Considering the four peaks analysed, the largest areas found are present in the cotyledon, followed by the seedling and leaf/stem.

Thus, there is a tendency to have polyphenols and, consequently, more catechin in cotyledon and seedling extracts than in leaf/stem. For the hypothesis of the presence of the epigallocatechin molecule, at 2922 and 2850 cm^{-1} , another band was assigned to this compound, which is 1366 cm^{-1} , due to the symmetrical stretching of CH₃ [24]. It was verified in (Fig. 3) that the area of this peak is also greater in the cotyledon extract.

When analysing the band range between 1646 cm^{-1} to 1044 cm^{-1} , we can evidence the possible

presence of flavonoids and, consequently, the possible participation of tannins in this identified range. These bands, according to the study carried out by Ramos et al. [29], demonstrated that there are links between nitrogen and hydrogen in this range; these compounds are found in proteins or alkaloids and may be directly linked to the presence of phenolic or nitrogen compounds in the oscillatory bands found. These bands obtained with the phenolic compounds can be correlated because they are linked to protein precipitation and free radical uptake. It is possible that the bands found may demonstrate the presence of these compounds since the basic unit of phenolic compounds is an aromatic ring attached to a functional hydroxyl.

The bands at 3009, 2850, 1438, 1366, 1313, 1226, 1050, 925 cm^{-1} were analysed according to Table 4, and the structural Friedelina molecule with its infrared spectrum (Fig. 4) obtained using a Friedelina pure KBr disk from data obtained from the literature. It is possible to observe the presence of this compound in the cotyledon extract.

It should be noted that the bands at 3009, 1313, 1226, 1050 and 925 cm^{-1} appear only in the cotyledon. Thus, from the infrared spectra data obtained from the analysed samples, there is a greater presence of terpenes, such as the Friedelina compound, and phenolics (tannins), such as the Epigallocatechin compound (main agents in the synergism of the treatment of gastric ulcers), in cotyledons than in the rest of the extracts analysed.

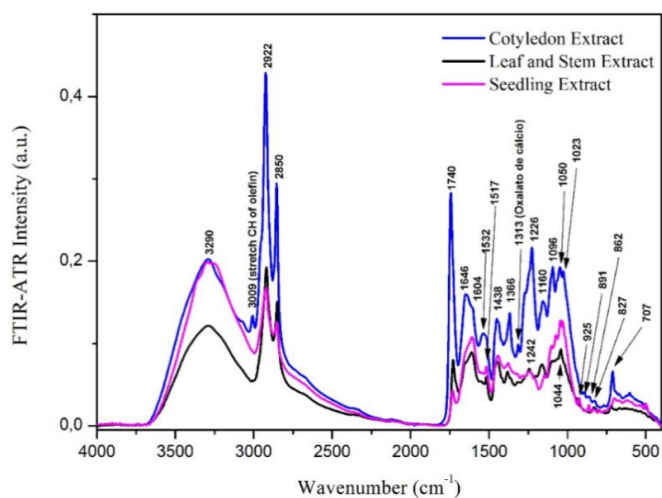


Fig. 2. Samples of Leaf/Stem, Seedlings and Cotyledon, obtained from the analysis of in the Fourier Transform FTIR infrared spectrometer with ATR of the *Maytenus ilicifolia*.

(Source: Author)

Table 4. Main bands obtained in the FTIR-ATR spectra of the samples (cotyledon, seedling and leaf/stem) of the *Maytenus ilicifolia* plant and functional groups associated with the spectra. The values of the respective wavelengths are described in cm^{-1}

L = Lipid; P = Protein; M = Mineral			
Cotyledon (cm^{-1})	Seedling (cm^{-1})	Leaf/Stem (cm^{-1})	Assignments
3290	3290	3290	L – $\nu(\text{C-H})$, $\nu(\text{C-H})$, aromatic or olefin [29]; O-H associated; strong, broadband, resulting from the polymeric association [30]
3009			L – $\nu(\text{=CH})$ [29]
2922	2922	2922	L – asymmetric $\nu(\text{C-H})$ [29]
2850	2850	2850	L – symmetrical $\nu(\text{C-H})$ [29]
1740	1740	1740	L – $\nu(\text{CO})$ carbonyl ester [29]
1646	1646	1646	C=C alkene; Amide [30]
1604			- NH_3 [30]
1532			P – N-H “dobra” (Amide II) [29]
	1517		P – N-H “dobra” (Amide II) [29]
1438	1438	1438	CH_2
1366	1366	1366	CH_3
1313			$\nu\text{S CO}_2$ (Calcium oxalate) [29]
	1242	1242	δCH_2 [29]
1226			P – NH “dobra” (amide III), O-H on the plan [29]; C-O aliphatic ethers [30]
1160			L–methyl ester, $\nu(\text{CO})$ [29]
1096			C – Amide OH, cellulose [29]
1050			P – resonance ring [29]
1023			M – PO_4^{3-} [29]
	1044	1044	P – resonance ring [29]
925			O-H; Broadband (angular deformation) of medium intensity, due to the angular deformation outside the C = O plane of carboxylic acids
891			$\text{R}_2\text{C}=\text{CH}_2$
862			C-H outside the plane
827			C – $\delta(\text{COC})$ [29]
707	707	707	C-H outside the plane

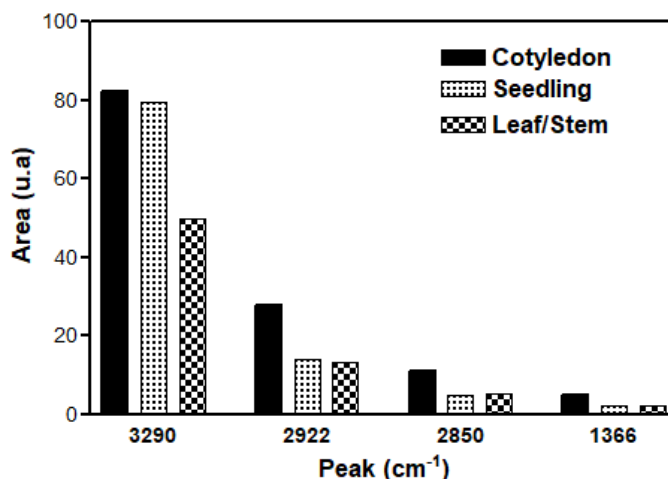


Fig. 3. Samples of leaf/stem, seedlings and cotyledon versus peak area at 3290 cm^{-1} , 2922 cm^{-1} , 2850 cm^{-1} and 1366 cm^{-1} . The areas were carried out with full calculation in the Origin 8.5 program (Source: Author)

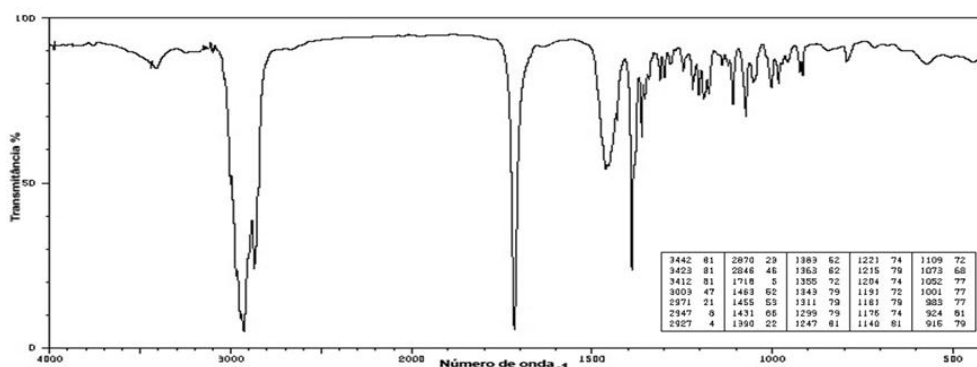


Fig. 4. Infrared Spectrum for Friedelina in KBr disk and its respective transmittance bands
(Source: https://sdbs.db.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi) accessed in July 2019

It is worth remembering that infrared spectroscopy - FTIR provides data indicative of the presence of molecules of organic compounds and phytocomplexes present in solid materials. However, with the data found and analysed using the database of the bibliography used in the research, it is possible to assume that the presence of the compounds found in the secondary metabolism of *Maytenus ilicifolia*, may be already present in the initial stages of plant growth as well as in the adult phase.

4. CONCLUSION

The results obtained by photoacoustic spectroscopy and spectroscopy analyses in the FTIR-ATR infrared region in lyophilized samples demonstrate that there is a chemical variation in the composition of the different samples of the *M. ilicifolia* plant.

With the results obtained in the photoacoustic spectroscopic analysis, it was possible to clarify the presence of chlorophylls, with their possible absorption points of light energy, assisting in the development and secondary metabolism of the plant for the formation of bioactive compounds, such as terpenes and flavonoids.

In the spectroscopy analyses in the FTIR infrared region with ATR, the presence of catechins in greater quantity in the cotyledon and seedling was demonstrated and bands that are suggestive of epigallocatechin were also observed. This has already been described in the literature only on the leaves of this plant. In the cotyledons, bands were observed which are attributed to the presence of friedelin, a compound that the studies analysed in the literature attributed to *M. ilicifolia* for the treatment of gastric pathologies.

In this way, the systematic cultivation in the laboratory to produce herbal medicines contributes in a positive way to the existence and sustainability of the species in the natural environment.

Characterising the compounds originating from the secondary metabolism present in the *Maytenus ilicifolia* plant using these spectroscopic techniques was based on directing and developing the use of cotyledons and seedlings as an alternative for use by the population, be it through artisanal production through planting by the community or industrially through chemical and/or pharmaceutical processing laboratories with technology and the ability to extract active compounds. Furthermore, the use of these techniques aims at not using solvents or chemical compounds in the characterization of compounds and is a sustainable alternative for analysis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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