IDENTIFICATION OF FUNCTIONAL GROUPS IN *MATOUREA AZUREA* ESSENTIAL OIL BY FTIR AND THEORETICAL IR SPECTRUM OF ITS MAIN TERPENES VIA DFT

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RESUMO: A espécie *Matourea azurea* é uma planta ornamental conhecida por seu óleo essencial, rico em mono- e sesquiterpenos com potenciais aplicações nas indústrias farmacêutica e cosmética. Neste estudo, técnicas experimentais e teóricas foram utilizadas para caracterizar o perfil químico de seus principais constituintes. A espectroscopia no infravermelho com transformada de Fourier (FTIR) foi empregada para identificar os grupos funcionais presentes no óleo essencial, enquanto a Teoria do Funcional da Densidade (DFT), utilizando o funcional B3LYP e o conjunto de base 6-311++G(d,p), foi aplicada para calcular o espectro infravermelho de seus terpenos majoritários. Os resultados indicaram a presença de grupos funcionais como aldeídos e álcoois, confirmada pelas bandas características observadas no espectro FTIR. Além disso, a identificação dos terpenos foi realizada com base em dados da literatura, e os espectros teóricos permitiram a identificação e atribuição das vibrações moleculares em β-copaen-4α-ol, viridiflorol, trans-pinocarveol, mirtenal e pinocarvona, proporcionando uma compreensão detalhada de suas interações vibracionais. Esses achados contribuem para a caracterização qualitativa da composição química da espécie e ampliam o conhecimento sobre os compostos bioativos presentes em seu óleo essencial.

PALAVRAS-CHAVE: espectroscopia; B3LYP; modos vibracionais; aldeído; álcool.

IDENTIFICATION OF FUNCTIONAL GROUPS *IN MATOUREA AZUREA* ESSENTIAL OIL BY FTIR AND THEORETICAL IR SPECTRUM OF ITS MAIN TERPENES VIA DFT

ABSTRACT: The species *Matourea azurea* is an ornamental plant recognized for its essential oil, which is rich in mono- and sesquiterpenes with potential applications in the pharmaceutical and cosmetic industries. This study employed both experimental and theoretical techniques to characterize the chemical profile of its main constituents. Fourier-transform infrared spectroscopy (FTIR) was utilized to identify the functional groups present in the essential oil. Additionally, Density Functional Theory (DFT), using the B3LYP functional and the 6-311++G(d,p) basis set, was applied to calculate the infrared spectrum of its major terpenes. The results revealed the presence of various functional groups, including aldehydes and alcohols, which were confirmed by the characteristic bands observed in the FTIR spectrum. Furthermore, the identification of terpenes was conducted based on existing literature. The theoretical spectra facilitated the identification and assignment of molecular vibrations in compounds such as β-copaen-4α-ol, viridiflorol, trans-pinocarveol, myrtenal, and pinocarvone. This provided a detailed understanding of their vibrational interactions. These findings contribute to the qualitative characterization of the chemical composition of Matourea azurea and enhance our knowledge of the bioactive compounds present in its essential oil.

KEY WORDS: spectroscopy; B3LYP; vibrational modes; aldehyde; alcohol.

INTRODUCTION

Essential oils are natural volatile compounds known for their high chemical complexity and intense biological activity. They are primarily produced by plants and stored in specialized structures such as glandular trichomes, secretory canals, or specific cells found in various plant organs, including leaves, flowers, fruits, seeds, bark, and roots. These metabolites mainly belong to the terpenoid and phenylpropanoid classes, which are derived from the mevalonic acid and shikimate metabolic pathways, respectively (Das and Prakash, 2023; Mengwen et al., 2024).

The composition of essential oils varies depending on the plant species, environmental conditions, and the plant's developmental stage. These substances play vital physiological roles, protecting herbivores and pathogens by acting as antimicrobial compounds and insect repellents. Additionally, essential oils are involved in both intra- and interspecific communication processes, influencing the attraction of pollinators and seed dispersers. In an ecological context, they can also affect interactions between plants, promoting allelopathy. In this mechanism, chemical compounds released by one plant either inhibit or stimulate the growth of other species. (Aqeel et al., 2023; Fernandes et al., 2024).

Essential oils have broad applications in the pharmaceutical, cosmetic, food, and perfumery industries due to their therapeutic, preservative, and aromatic properties. Their antimicrobial, antioxidant, and anti-inflammatory activities have garnered increasing interest for use in herbal medicines and natural antimicrobial formulations (Kakouri et al., 2022; Liu et al., 2024). Beyond industrial applications, many traditional communities use essential oils in their medicinal, ritualistic, and cosmetic practices. Indigenous peoples and rural populations employ these substances in infusions, ointments, and baths, drawing on ancestral knowledge to treat ailments, repel insects, or enhance spiritual and energetic balance. This traditional knowledge has been increasingly valued, inspiring scientific research aimed at investigating and formulating sustainable products (Sumarni et al., 2022).

The species selected for this study is Matourea azurea (Linden) Colletta; V.C. Souza, frequently referenced in literature by its synonyms Achetaria azurea (Linden) V.C. Souza or Otacanthus azureus (Linden) Ronse. Belonging to the Plantaginaceae family, this species is native to southeastern Brazil but has demonstrated significant adaptability, leading to its introduction in northern Brazil and its cultivation in various parts of the world, including the United States, France, India, and Japan. M. azurea exhibits heliophile growth behavior, a high-water demand, and efficient propagation through asexual reproduction. Its essential oil is predominantly composed of mono- and sesquiterpenes, with β-copaen-4α-ol and myrtenal as its main constituents. Additionally, previous studies have highlighted its antifungal properties and inhibitory activity against Leishmania amazonensis, suggesting potential pharmaceutical applications (Andrade et al., 2006; Serudo et al., 2013; Houël et al., 2014).

Fourier Transform Infrared Spectroscopy (FT-IR) is an analytical technique used to identify and characterize chemical compounds based on molecular vibrations associated with chemical bonds. In the context of essential oils, FT-IR enables the qualitative and quantitative analysis of their components, identifying characteristic functional groups such as C=O, O-H, C-H, and C=C bonds present in terpenoids, phenylpropanoids, and other classes of secondary metabolites. The technique also helps verify oil purity, detecting adulterations or variations in chemical composition resulting from environmental or processing factors (Agatonovic-Kustrin et al., 2020).

Thus, the combination of FT-IR spectroscopy and theoretical calculations based on Density Functional Theory (DFT) provides a robust approach for the structural characterization and understanding of the spectroscopic properties of essential oils. In the present study, these techniques are applied to the species M. azurea to achieve the vibrational identification of its primary terpenes, including viridiflorol, β-copaen-4α-ol, trans-pinocarveol, myrtenal, and pinocarvone. Computational modeling enables the precise assignment of spectral bands for these compounds, facilitating the correlation between experimental and theoretical data and reducing ambiguities in spectral interpretation.

MATERIALS AND METHODS

Fourier Transform Infrared Spectroscopy (FT-IR)

The FTIR equipment used for analysis was the IRAffinity-1S, manufactured by SHIMADZU, equipped with an ATR (Attenuated Total Reflectance) cell with a zinc selenide crystal. The spectral resolution used was 16 cm^{-1}, with a scanning range from 4000 to 500 cm^{-1}. This analysis was conducted to identify the functional groups present in the essential oil of M. azurea, harvested in August 2023. The samples were analyzed at the Central Laboratory of Chemical Analyses (CAQ/QAT) of the School of Technology at the University of the State of Amazonas (UEA).

Molecular Optimization

The optimization of the most abundant molecules in the essential oil extracted from *M. azurea* leaves was performed using the free software Avogadro. The molecular structures were obtained from SMILES (Simplified Molecular Input Line Entry System) data available in PubChem. The force field used to generate the *INP file was MMFF94s (Merck Molecular Force Field 94s). To improve efficiency, this process started with force fields of increasing accuracy, culminating in MMFF94s, which provided optimization with the lowest energy, indicating the molecule’s ground state. The order of force fields, based on increasing sophistication and typical accuracy in molecular modeling, was as follows: GHEMICAL, UFF (Universal Force Field), GAFF (General Amber Force Field), MMFF94 (Merck Molecular Force Field 94), and finally MMFF94s. The optimization algorithm used was the Conjugate Gradient method, with 100 steps per update (Hanwell et al., 2012).

Density Functional Theory

The chosen functional, selected based on a balance between computational cost and accuracy, was B3LYP (Becke, 3-parameter, Lee-Yang-Parr) with the 6-311++G(d,p) basis set. The software used was Orca 6.0, with the input file containing the following syntax:

```
! B3LYP OPT FREQ 6-311++G(d,p) TightSCF PAL6 RIJCOSX
```

Where: B3LYP: Functional used; OPT: Indicates that a geometry optimization was performed; FREQ: Requests a frequency calculation after geometry optimization to verify if the optimized structure is an actual minimum and to obtain vibrational properties; 6-311++G(d,p): Specified basis set; TightSCF: Configures stricter convergence criteria for the Self-Consistent Field (SCF) cycle PAL6: Utilized 6 CPU cores for parallel computation RIJCOSX: Approximation technique to accelerate exchange integral calculations.

DFT is a combination of methods that provides an approximation for calculating the electronic energy of a molecule, taking into account quantum mechanical effects. In this context, B refers to Becke's exchange functional, which is a correction to the Hartree-Fock exchange functional. 3LYP represents a combination of three functionals: the Hartree-Fock exchange functional, the Becke (B) exchange functional, and the Lee-Yang-Parr (LYP) correlation functional (Becke, 1993).

RESULTS AND DISCUSSION

Fourier Transform Infrared Spectroscopy (FT-IR)

The graph in Figure 1 presents the transmitance bands for the essential oil of *M. azurea*. In the wavelength region of 2927 cm^{-1} and 2872 cm^{-1}, the stretching of the C-O bond characteristic of the aldehyde chemical group is observed. The presence of the doublet allows the distinction of other groups (Silverstein et al., 2014; Pavia et al., 2015). The "fingerprint" region is located approximately between 400 cm^{-1} and 1500 cm^{-1}. It is highly characteristic for each molecule, meaning that even compounds with the same functional groups can have distinct spectra in this region. It is used for the unique identification of essential oils and other substances.

At 1460 cm^{-1}, there is absorption due to CH scissoring (bending) vibrations near the carbonyl group. The peaks in the 850–920 cm^{-1} range correspond to the absorption of methylene groups. Near 875 cm^{-1}, β-pinene absorption is detected due to the ring structure strain with the exocyclic CH_2 group. This band may also indicate the presence of myrcene; however, it was not identified in the chromatography analysis, despite being present in other chemotypes of *M. azurea* (Agatonovic-Kustrin et al., 2020).

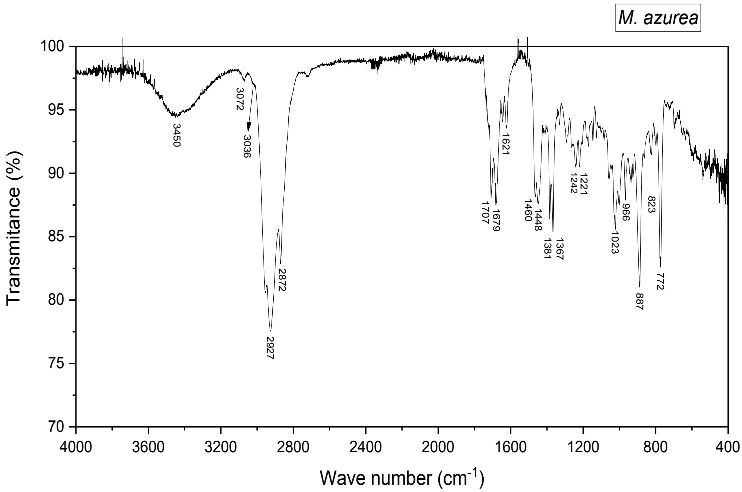

Figure 1: FTIR – Spectrum of the essential oil of *M. azurea*.

The peak at 1448 cm^{-1} may result from the overlap of CH_2 deformation with asymmetric CH_3, where intensity is proportional to the quantity of these two groups. The peaks at 1679 and 1621 cm^{-1} correspond to the stretching vibration of the aldehyde carbonyl group. The peak near 1450 cm^{-1} indicates the presence of alcohols, with absorption corresponding to the bending vibration of C-OH (Li et al., 2013).

Each type of bond has its natural vibration frequency. Since two identical bond types in different compounds exist in slightly different environments, the infrared absorption patterns of two molecules with other structures are never identical (Pavia et al., 2015). In homogeneous solutions such as essential oils, the spectra of individual components may show overlapping and mixing of various vibrational modes (Agatonovic-Kustrin et al., 2020). Thus, FTIR can be used as a technique for differentiating plant species based on patterns in the fingerprint region. The C-O stretching and O-H bending are not independent vibrational modes, as they may couple with vibrations from adjacent groups (Silverstein et al., 2015). The observed doublet in the 2860 cm^{-1} to 2700 cm^{-1} range of an aldehyde is a result of Fermi resonance. In this case, the band detected in Figure 2 likely corresponds to myrtenal, the most abundant molecule in *M. azurea* essential oil. The second band appears when the C-H stretching vibration couples with the first harmonic of the medium-intensity aldehyde C-H bending vibration, which occurs in the 1400 cm^{-1} to 1350 cm^{-1} range. The intensity at 1680 cm^{-1} suggests a longer conjugation system for the C=O bond.

Although the essential oil of *M. azurea* contains hydroxyl-bearing compounds, and the characteristic broad and intense band is frequently reported in the literature, factors such as molecular symmetry may influence vibration behavior. The peak's behavior in this region can be explained by harmonic bands, which occur when two bands with strong electric dipoles overlap. Additionally, concentration effects play a role, as even though most of the essential oil composition consists of alcohols like β-copaen-4α-ol and trans-pinocarveol, these substances do not entirely overshadow the remaining fraction. However, De Pooter et al. (1989) mentioned in their study a characteristic broad O-H absorption band when working with purified β-copaen-4α-ol from *M. azurea*, confirming that the newly identified molecule was an oxygenated sesquiterpene, which enabled its identification.

Calculated IR spectrum and vibrational modes

Using DFT at B3LYP with the 6-311++G(d,p) basis set, it was possible to computationally generate the infrared spectrum of the isolated elements representing the most prominent areas identified in the chromatogram from previous work, as shown in Figure 2 (Souza, 2024).

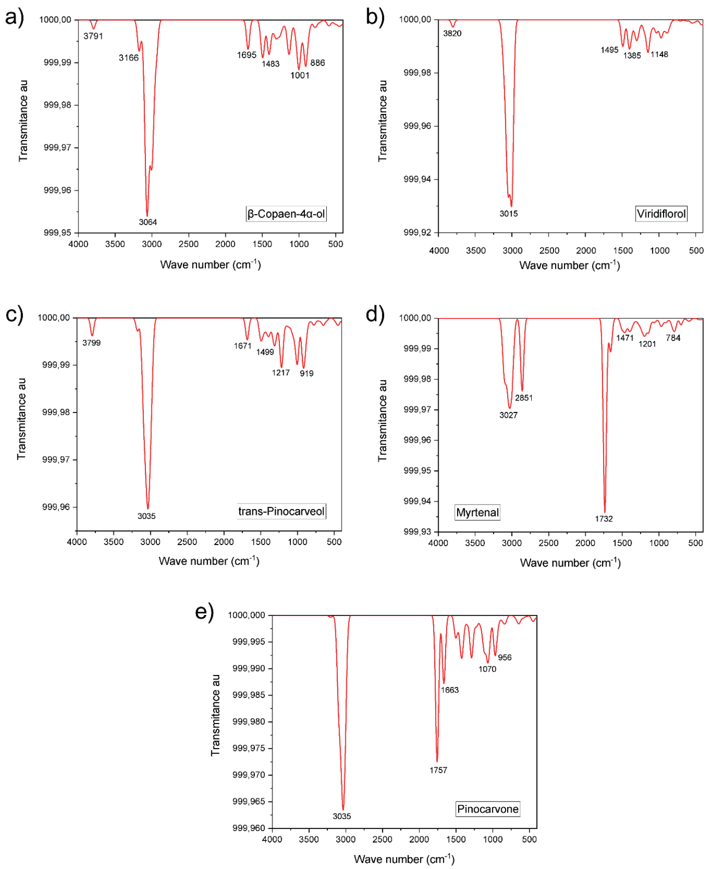

Figure 2. IR spectrum calculated at B3LYP, 6-311++G(d,p) of the main terpenes found in *M. azurea* essential oil. a) β-copaen-4α-ol; b) viridiflorol; c) trans-pinocarveol; d) myrtenal; e) pinocarvone.

The data for the wavenumber variable was corrected using a scaling factor of 0.9951 (Zochedh et al., 2022). The data in Table 1 describes the type of vibration corresponding to the peak intensity of the infrared spectrum, as visualized in Chemcraft software. The absence of imaginary frequencies demonstrates that the molecular geometry optimization calculation was successful, representing the molecule’s ground state.

Molecule	Wave Number (cm^{-1})	Vibrational mode
β-Copaen-4α-ol	289,44	δ(O-H)
	915,73	δ(H-C-H)
	1400,11	δ(H-C-H)
	1700,96	δ(H-C-H) (scissoring)
	3077,77	v(H-C-H)
	3812,27	v(O-H)
Viridiflorol	300,62	δ(O-H)
	1155,49	δ(H-C-H)/ δ(C-H)
	1300,73	δ(O-H)/ δ(C-H)
	3029,85	v(C-H)
	3821,01	v(O-H)
trans-Pinocarveol	345,03	δ(O-H)/ δ(H-C-H)
	919,9	δ(H-C-H)
	1321,02	δ(H-C-H)
	1401,53	δ(H-C-H)
	3049,84	v(C-H)
	3812,39	v(O-H)
Pinocarvone	73,06	ω(H-C-H)/ ρ(H-C-H)
	353,85	ρ(H-C-H)/ δ(C=O)
	1673,48	δ(H-C-H) (scissoring)
	1766,09	v(C=O)/ δ(H-C-H) (scissoring)
	3049,51	v(C-H)
	3222,57	v(C-H)
Myrtenal	784,49	δ(C=O)
	1207,31	δ(H-C-H)
	1744,61	v(C=O)
	2875,06	v(C-H)
	1666,07	v(C=C)/ ω(C-H)*
	2875,06	v(C-H)
	3099,3	v(C-H)

δ = Bending); v = Stretching; ω = Wagging; ρ = Rocking.

Table 1. Vibrational modes in the IR spectrum of terpenes from *M. azurea* essential oil.

Experimental data often differ because infrared absorption occurs in molecules with different energy states beyond the fundamental state, as well as due to differences in the state of matter (Costa et al., 2020). Additionally, interactions between the material and solvents or other molecules may result in variations in the absorption of infrared light.

It is interesting to note that the O-H stretching in tertiary alcohols appears in regions with higher wavenumbers and has a similar shape. The stretching of trans-pinocarveol, a secondary alcohol, exhibits a slightly different band compared to tertiary alcohols, with greater intensity. Under experimental conditions, this O-H vibration may appear at lower wavenumbers.

Based on the calculated data, it can be stated that the strong peaks in higher wavenumber regions observed in the experimental spectrum of *M. azurea* essential oil result from the overlapping vibrations of different functional groups.

The objective of investigating vibrational modes is to determine which vibrational motions contribute to each of the experimental bands (Udayappan et al., 2024). The wavenumber values differ from the graphs because they correspond to the values before standardization.

Alcohols exhibit stretching vibrations in the functional group at higher wavenumber regions, around 3800 cm^{-1}, and bending vibrations at lower wavenumber regions. For example, β-Copaen-4α-ol has this bending peak at 289.44 cm^{-1}. As mentioned at the beginning of this section, myrtenal presents C=O stretching at 1744 cm^{-1}, and around 784 cm^{-1}, there is bending in these atoms. The aldehyde functional group in myrtenal also exhibits C-H bending vibration at 2875 cm^{-1}.

CONCLUSION

The functional groups identified in the essential oil of *M. azurea* are consistent with the presence of terpenes reported in previously published studies, reinforcing the characteristic chemical composition of this genus. Furthermore, Density Functional Theory (DFT) proves to be a powerful tool in the interpretation of these vibrational bands, allowing for a more precise assignment of the experimentally observed peaks. However, to achieve a better correlation between theoretical and experimental data, it is necessary to perform spectroscopic tests with isolated terpenes, enabling a direct comparison with the calculated results. Additionally, calculating vibrational frequencies under different solvation conditions is essential to account for medium effects and obtain more realistic predictions of the bands observed in experimental FTIR.

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