Geographic modulation of the phytochemical profile of Tithonia diversifolia (Hemsl.) A. Gray

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Abstract

The chemical profile and temporal stability of hydroalcoholic extracts from Tithonia diversifolia collected in five locations in Espírito Santo, Brazil, were investigated. Samples were gathered during vegetative (2014) and reproductive (2015) stages, with a follow-up collection in 2024 for two sites (UFES-Vitória and Santa Teresa). Analysis using Fourier Transform Infrared Spectroscopy (FTIR), Mass Spectrometry (ESI-TOF), and chemometrics (PCA, HCA) was performed. FTIR confirmed functional groups characteristic of alcohols, phenols, and lipids. Multivariate analysis indicated clear geographical clustering, with samples from Santa Teresa showing the greatest chemical divergence and variability over time. ESI-TOF enabled the putative annotation of diverse bioactive metabolites, including hydroxycinnamic acids (e.g., caffeoylquinic acid), flavonoids, sesquiterpenes (tagitinin A), and fatty acids. After a decade of storage, qualitative composition remained largely stable, except in Santa Teresa, where quantitative shifts were more evident. The results demonstrate that the chemical composition of T. diversifolia exhibits significant spatial and temporal variation, influenced by geographical and phenological factors.

1 Introduction

The rising incidence of chronic and degenerative diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions, has driven the search for natural compounds with therapeutic properties, especially those derived from medicinal plants. In this context, medicinal plants represent an invaluable source of structurally diverse secondary metabolites capable of modulating key molecular and cellular pathways involved in oxidative stress, inflammation, and genomic instability. Among promising species, Tithonia diversifolia (Asteraceae), commonly known as 'tree marigold' or 'Mexican sunflower', stands out. Its extracts have been associated with antioxidant, anti-inflammatory, antimicrobial, and, more recently, antiproliferative activities (Benkhaira et al. 2022). These properties are closely linked to its chemical profile and, consequently, to secondary metabolites such as sesquiterpene lactones, flavonoids, phenolic acids, and hydroxycinnamic derivatives, which are known to modulate redox balance, inflammatory mediators, cell cycle progression, and signaling pathways involved in apoptosis and cellular proliferation.

Despite the recognized bioactivity ofT. diversifolia, the chemical variability influenced by geographical, phenological, and environmental factors remains underexplored, particularly regarding the temporal stability of extracts and its implications for biological activity. The chemical profile of medicinal plants is dynamic and may vary significantly according to environmental conditions, edaphoclimatic factors, and plant developmental stage, directly impacting metabolite concentration and qualitative composition. Understanding these variations is essential for extract standardization, reproducibility of pharmacological effects, and future therapeutic applications.

Furthermore, integrating advanced analytical techniques like infrared spectroscopy (FTIR) and mass spectrometry (ESI- TOF) with chemometric analyses (PCA, HCA) can provide a systemic understanding

of the chemical composition, stability, and pharmacological potential of these extracts. FTIR enables rapid functional group fingerprinting, whereas ESI-TOF- MS allows high-resolution molecular profiling, and when combined with multivariate statistical tools, these techniques enhance discrimination of metabolic patterns associated with geographical and phenological influences (Chidi et al. 2017; Stark et al. 2013).

Given this context, this work aimed to analyze the chemical composition and compound preservation in hydroalcoholic extracts of Tithonia diversifolia obtained by exhaustive maceration of leaves. The plants were collected from five locations in Espírito Santo, Brazil, during vegetative (2014 and 2024) and reproductive (2015) stages. The chemical profiles were

correlated with phenological and geographical variables to evaluate metabolic variability and extract stability, contributing to the standardization and pharmacological validation of this medicinal species.

2 Material and methods

2.1 Plant material and extraction

The evaluated material belongs to the Extract Bank of the Plant Genetics and Toxicology Laboratory at the Federal University of Espírito Santo (UFES), Brazil. Hydroalcoholic extracts of Tithonia diversifolia were obtained from the aerial parts of adult plants collected randomly across five localities in Espírito Santo State, Brazil: Colatina (COL; 19°30'08.2" S, 40°36'40.7" W), Muniz Freire (MF; 20°28'09.9" S, 41°24'14.7" W), Santa Teresa (ST; 19°55'38.9" S, 40°35'23.0" W), Viana (VIA; 20°25'23.2" S, 40°28'37.3" W), and Vitória (VIT; 20°16'30.5" S, 40°18'17.5" W). Collections were carried out during both the vegetative (September 2014) and reproductive (April 2015) phenological stages. The plant material was taxonomically identified by Dr. Jimi Naoki Nakajima, and representative voucher specimens (VIES 35297 and VIES 35298) were deposited at the VIES Herbarium (UFES). Extracts were prepared by Dr. Irany Rodrigues Pretti via exhaustive maceration in 70% ethanol at room temperature, protected from light, according to Pretti et al. (2018).**Figure 1**: Geographic distribution of sampling sites in Espírito Santo State, Brazil, highlighting the municipalities where plant material was collected. Adapted from Geobases – Integrated System of Geospatial Databases of the State of Espírito Santo (2026).

2.2 Infrared Spectroscopy

To infer the current composition and chemical profile of the plant extracts, Fourier Transform Infrared Spectroscopy (FTIR) was employed using a Cary 360 FTIR device (Agilent Technologies, USA). Each extract aliquot was individually and directly deposited onto the apparatus to obtain the spectrograms. The collected data were analyzed using OriginPro® 2024 software, and the results were interpreted and compared with one another.

2.3 Multivariate Analysis

Spectral data were pre-processed using the PLS-Toolbox extension available in MATLAB software, version R2024B (The MathWorks Inc.; Eigenvector Research Inc.). To improve data quality, smoothing and scatter correction methods were applied. Smoothing was performed using the Savitzky–Golay algorithm (order 2, 15-point window) to reduce noise in the spectra. Additionally, the data were mean-centered (MC), and Multiplicative Scatter Correction (MSC) was applied to adjust for possible baseline scattering variations.

2.4 Electrospray Ionization Mass Spectrometry (ESI- TOF)

The analyses were carried out on a maXis impact Q-TOF mass spectrometer (Bruker Daltonics) equipped with an electrospray ionization (ESI) source. The system operated alternately in positive and negative ion modes. Samples, prepared at a concentration of mg/ml in methanol, were injected directly at a flow rate of 3 µL/min. The ionization parameters were set as follows: capillary voltage of 4500 V (positive mode) or 3200 V (negative mode), nebulizer gas pressure of 1.0 Bar, and desolvation temperature of 200°C. Spectra were acquired in the m/z range of 50 to 1500, with a resolution of approximately 60,000 (defined by full width at half maximum, FWHM, at m/z 400). Data processing was performed using DataAnalysis 5.0 software (Bruker Daltonics), metabolite annotations were considered putatively assigned when the mass error was equal to or less than 15 ppm. Furthermore, a mass error of ± 15 ppm was allowed, considering the following positive adducts ([M + H], [M+NH4], [M + Na], [M + K], [2M + H]) and negative adducts ([M-H], [M-H_2O-H], [M-Cl], [2M + H]).

2.5 Statistical Analysis

For all experiments, differences were considered statistically significant when the α-value was less than 0.05 ($p < 0.05$). For chemical analyses, FTIR spectra were processed and generated using OriginPro® 2024 software. Multivariate analyses, including Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA), were performed in MATLAB software using the PLS-Toolbox resources. Mass spectrometry data analysis was conducted using Compass DataAnalysis 4.0 software. Metabolite annotation was performed based on accurate mass measurements, considering the lowest mass errors (ppm), comparison with previously reported compounds in the literature, and information available in the PubChem database.

3 Results and Discussion

3.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR analysis of Tithonia diversifolia extracts contributed to the characterization of their organic chemical composition. The interpretation of the spectra was based on previously published studies describing the assignment of absorption bands and functional groups in infrared spectroscopy (Lopes and Fascio, 2004; Carballo et al. 2008; Movasaghi et al. 2008; Pavia et al. 2010; Oliveira et al. 2016; Thombare et al. 2023). Based on these references, absorption bands within the spectral range of 4000–250 cm^{-1} were assigned to their corresponding functional groups. From the analyzed spectra of the leaf

extracts of Tithonia diversifolia from the five localities in the years 2014 and 2015, it can be observed that all extracts displayed broad absorption bands between 3100–3400 cm^{-1}, attributed to O–H bond stretching. These characteristics belong to the group of alcohols, phenols, and possible flavonoids (Table 1). Furthermore, the broadening of the band in this region, observed in Fig. 2A, may be associated with the presence of water and/or increased hydrogen bonding interactions, possibly related to the storage time of the extracts.

Table 1
FTIR bands and corresponding functional groups identified in the *Tithonia diversifolia* samples.

Item	Wavenumber (cm^{-1})	Functional group / Chemical assignment
1	3320	O–H stretching (alcohols, phenols, polyols)
2	2900–2850	C–H stretching (aliphatic; presence of fatty acids such as octadecanoic acid)
3	2240	Aliphatic C–H stretching (lipids and hydrocarbons)
4	1740–1690	C = O stretching (carbonyl groups of aldehydes, ketones, and lactones)
5	1692	N–H stretching (primary amines)
6	1615	C = C stretching (aromatic ring; phenols and terpenoids)
7	1555	C–N stretching (amines)
8	1194	C–N stretching (aromatic amines)
9	1009	C–H stretching (polysaccharides and glycosylated compounds)

Source: Authors.

The region near 2920–2850 cm^{-1}, observed in Figs. 2A and 2B, corresponds to the C–H stretching of aliphatic chains, suggesting the presence of lipid constituents. Although there is variation among localities over the years, the peaks remain in the same spectral regions, indicating the presence of similar chemical compounds or a similar chemical composition, with differences in transmittance intensity associated with variations in the relative abundance of the constituents (Pavia et al. 2010).

Another analysis range identified in both images is 1600–1500 cm^{-1}, which showed bands related to C = C stretching of aromatic rings and N–H stretching of amides, reinforcing the contribution of phenolic compounds and proteins/nitrogen derivatives. Previous studies of this species and the Asteraceae family highlight that its main chemical constituents belong to the classes of sesquiterpene lactones (SLs), flavonoids, and phenolic compounds derived from trans-cinnamic acid (Rolnik and Olas 2021).

Between 1400 and 1000 cm^{-1}, a set of intense bands attributed to C–O vibrations of polysaccharides, alcohols, and ethers was observed a region strongly associated with the presence of carbohydrates and

glycosides. Thus, it was found that most samples showed similar profiles in terms of band positions, albeit with differences in absorbance intensity.

It is important to emphasize that the intensity of the peaks presented is crucial for sample characterization, since FTIR spectroscopy is predominantly qualitative in nature and widely used for the identification of functional groups. However, as described by (Pavia et al. 2010), the technique can provide semiquantitative information based on the variation in band intensity, provided that the samples are analyzed under the same experimental conditions. In this way, bands with higher intensity may indicate a greater relative contribution of certain chemical groups, suggesting that although the qualitative composition is preserved, variations occur in the relative abundance of functional groups among the samples.

To evaluate the chemical stability of the extracts over time, an additional collection of plant material was carried out in 2024 from the localities of Santa Teresa and UFES. The selection of these two sites considered logistical criteria, such as proximity and accessibility. The newly obtained extracts were directly compared with those prepared from samples collected ten years earlier, enabling an analysis of the chemical profile after 10 years of storage (Fig. 3).

The analysis of the extract from UFES plants showed overlapping spectra across the years, with minimal variations in band shape and intensity. This spectral proximity suggests that the composition present in the samples remained largely unchanged compared to the extracts produced 10 years earlier, unlike the behavior observed in SANT.

The characteristic absorption bands of the species, including O–H (3300–3400 cm^{-1}), aliphatic C–H (2900–2800 cm^{-1}), and C = C (1700–1600 cm^{-1}), remained consistent over the years, indicating that functional groups associated with alcohols, phenols, carbonyl compounds, amines, and polysaccharides were largely preserved in the extracts (Movasaghi et al. 2008; Pavia et al. 2010). These findings suggest that the overall chemical composition remained relatively stable throughout the storage period. It is worth noting that Tithonia diversifolia extracts contain several classes of bioactive metabolites, including terpenoids, phenolic compounds, amines, and fatty acids, which have been associated with a wide range of biological activities. The comparative analysis of the FTIR spectra of the 2014 and 2015 extracts, however, revealed a stable spectral profile over time. This stability suggests that these compounds did not undergo significant chemical modifications or degradation during the studied storage period.

In contrast, in the extract from Santa Teresa, corresponding to the year 2024, significant variations in transmittance were observed over the years, especially in the characteristic O–H, aliphatic C–H, and carbonyl group regions, indicating consistent physicochemical changes in the molecular composition of the samples. The broad absorption band at 3300–3400 cm^{-1}, which was more intense in the 2015 samples, suggests a greater relative abundance of hydroxyl-containing compounds, including polyols, alcohols, and phenolic compounds. These functional groups are characteristic of phenolic metabolites and are closely associated with the antioxidant properties of plant extracts due to their ability to

participate in redox reactions and hydrogen-bonding interactions (Movasaghi et al. 2008; Pavia et al. 2010). The fluctuations observed in the bands at 2900–2800 cm^{-1} and in the region of 2240 cm^{-1} reflect variations in the proportion of aliphatic C–H, consistent with lipid compounds, including long-chain fatty acids such as octadecanoic acid, known for its antibacterial and cosmetic properties.

Furthermore, significant changes also occurred between 1700– 1500 cm^{-1}, regions associated with C = O vibrations, aromatic C = C, and N–H/C–N of amines, corroborating the presence of carbonyls, lactones, terpenoids, and amines. These functional groups are related to biological activity and described as responsible for antimicrobial and stabilizing properties in plant systems, as reported in Tithonia diversifolia (Ogundare 2007; Anthoney et al. 2016).

Within this general context, the comparative analysis between the production years of the Tithonia extracts collected in Santa Teresa revealed that, although SANT4 and SANT5 showed relatively similar spectral profiles, the SANT24 sample exhibited a distinct modification in band intensity across almost the entire spectrum. This change, evident in the 1000– 1200 cm^{-1} region, may be associated with polysaccharides and glycosylated compounds, and additionally, the band near

1740 cm^{-1} is related to C = O stretching of phenolic and carboxylic compounds. These changes suggest alterations in the profile of structural and secondary metabolites. One possible hypothesis for these differences is related to the vegetative stage of the plant at the time of collection in 2024, a phase during which active translocation of compounds and chemical elements derived from its metabolism essential for preparing the reproductive cycle occurs (Taiz et al. 2017).

This can be corroborated by previous results from Pretti et al. (2018).from the same research group, which reinforce the interpretation by demonstrating that the Santa Teresa population exhibits lower genetic similarity compared to the other localities, a characteristic compatible with abundant sexual reproduction and the great diversity of pollinating insects that act on T. diversifolia. This higher genetic variability may contribute to the metabolic fluctuations observed over the years, including the increase in phenolic compounds and antioxidant activity in the vegetative stage, as also recorded in previous studies by Pretti and collaborators (2018).Thus, the spectral variations observed in Santa Teresa over time reflect not only environmental and phenological modifications but also the inherent genetic dynamics of the population, resulting in a more diversified and unstable molecular profile when compared to the other analyzed localities.

3.2 Multivariate Analysis

To complement the FTIR characterization and verify the level of chemical similarity among the different localities, a chemometric analysis of the obtained results was performed using Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA). The multivariate analysis integrated the acquired data, simultaneously considering the multiple experimental variables involved: the PCA and HCA. The HCA revealed the formation of distinct groups among the samples of Tithonia diversifolia, highlighting some characteristics related to geographical origin (Fig. 4).

The dendrogram structure clearly shows that samples from the same locality tend to cluster together, indicating a common chemical profile within each region. This is evident in the close groupings among samples from UFES, Muniz Freire, and Viana (UFES4, UFES5, UFES2, MUF4, MUF5, VIA4, VIA5) and among those from Colatina (COL4, COL5), suggesting that plants collected within the same geographical area share a similar metabolic profile. However, the Santa Teresa sample from 2024 (SANT24) stood out as the most distant group from all others, suggesting chemical differences compared to the remaining samples, which corroborates the FTIR analysis previously discussed. A possible hypothesis for this behavior is that the 2024 collection was conducted at the site based on general directions, in addition to being located adjacent to a high-traffic highway, which may have influenced the results from samples derived from distinct individuals or even different microenvironments within the locality. It is known that environmental stressors of anthropogenic origin, such as pollution and soil alterations, can act as abiotic stress factors in plants. For example, a

study with Mentha piperita collected from areas with different levels of vehicular pollution observed marked variations in essential oil composition and antioxidant activity, demonstrating how anthropogenic stress can modulate secondary metabolism (Gharib et al. 2021). Such stressors have the potential to modulate the biosynthetic pathways of secondary metabolites, altering the production and accumulation of defense compounds. As reported by Asiminicesei et al. (2024), exposure to heavy metals, which are frequently associated with anthropogenic pollution, induces oxidative stress in medicinal plants, leading to the overproduction of reactive oxygen species (ROS) and the dysregulation of metabolic pathways involved in the biosynthesis of bioactive compounds, including polyphenols, alkaloids, and flavonoids.

An example of this mechanism was documented by Galal and Shehata (2015) in a study with Plantago major, a medicinal plant that, when grown in soils contaminated by metals derived from vehicular traffic, not only accumulates high levels of these contaminants but also reconfigures its translocation pattern between roots and shoots as a tolerance strategy. Such physiological reconfiguration, aimed at tolerating stress, compromises not only the plant's adaptive capacity but also its therapeutic properties, either through possible alterations in the bioactive profile or through direct contamination of plant material with toxic elements.

On the other hand, PCA allowed visualization of the organization and degree of similarity among the chemical profiles of the Tithonia diversifolia extracts, highlighting how each locality exhibits distinct patterns or specific clusters along the principal components (Fig. 5).

The first two components jointly explained 84.6% of the total variance in the data (PC1 = 67.41%; PC2 = 17.18%), indicating that the model robustly represents the observed variation. Thus, the representation closely approximates the multidimensional reality of the data, suggesting that residual insignificant information is low, which enhances the robustness of the analysis. From the graph analysis, a consistent clustering of the localities was observed: Colatina, Muniz Freire, UFES, and Viana. This result may suggest uniformity and proximity in the chemical composition of the analyzed samples, forming a

cohesive group. In contrast, the Santa Teresa samples (SANT4 and SANT24) showed greater separation both from the other localities and from each other, positioning themselves in distinct regions of the graph, which suggests a differentiated chemical composition and greater variability within the locality. This contribution is consistent with the quantitative data from the study, which attributes to Santa Teresa the highest levels of phenols, flavonoids, and tannins, as well as the greatest antioxidant activity among all evaluated populations (Pretti et al. 2018). Therefore, the distinct clustering observed in HCA and PCA analyses is not merely a statistical result but represents an interaction between the genotype of this population and the specific conditions of its habitat (high altitude, lower temperatures, and micronutrient-rich soil).

According to Pretti et al. (2018), RAPD (Random Amplified Polymorphic DNA) analysis revealed that Santa Teresa has low genetic similarity (0.12) with the other populations, indicating that its clustering is not correlated with geographical proximity but rather with specific intrinsic and environmental factors. These results suggest that geographical and/or phenological factors may be associated with the observed chemical heterogeneity, which is particularly notable in the case of the Santa Teresa samples, as also demonstrated in other analyses such as HCA and FTIR.

3.3 ESI-TOF Analysis

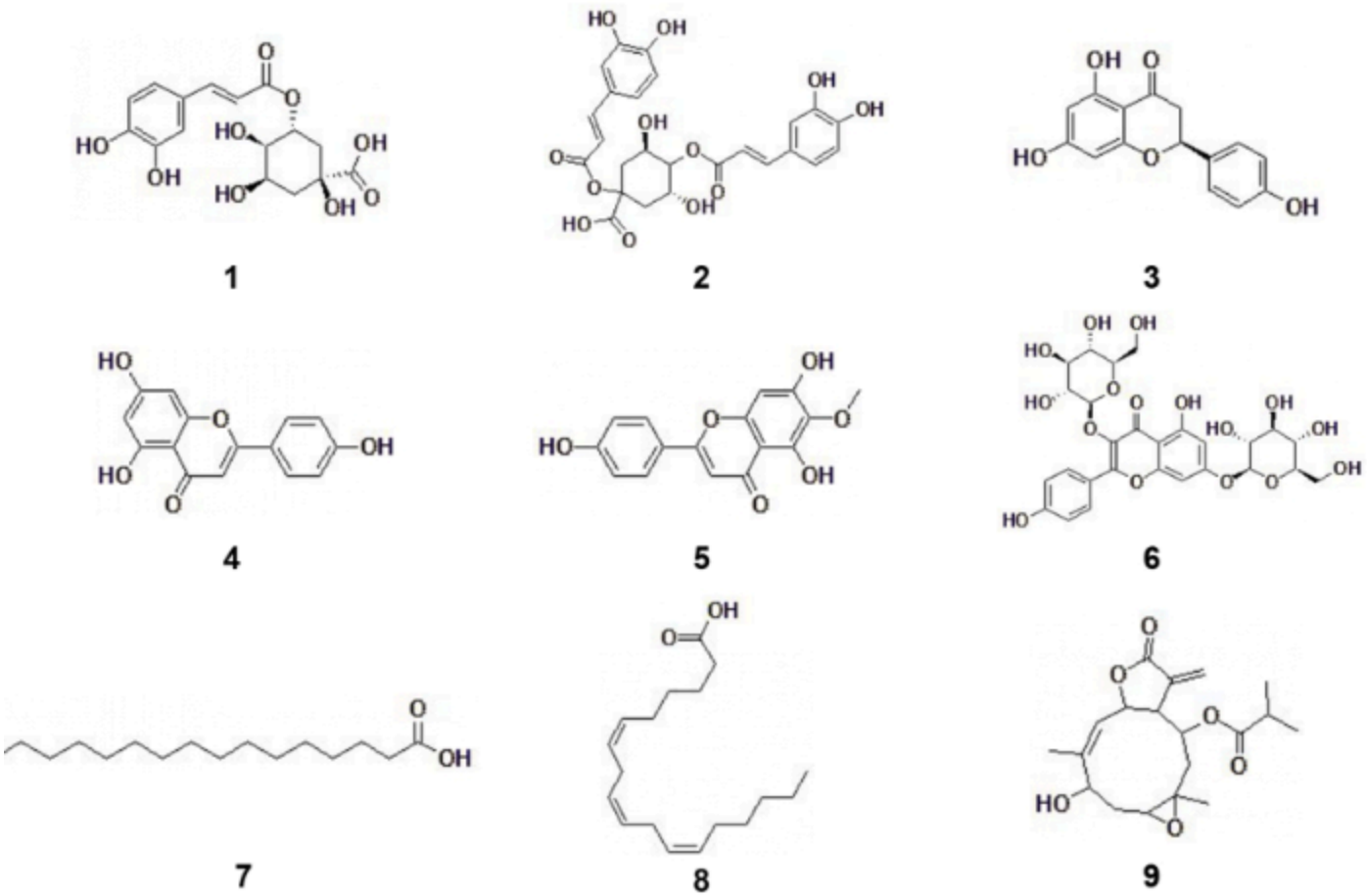
Mass spectrometry analysis of Tithonia diversifolia extracts enabled the tentative annotation and characterization of the major metabolites present in the samples, revealing molecular features tentatively assigned to different chemical classes. The interpretation of the detected ions was based on previously reported studies (Araújo et al. 2021; Kerebba et al. 2022; Gallon et al. 2019; Wang et al. 2025; Paula, 2013), which supported the putative annotation of metabolites and the comparison of chemical profiles among the different localities. Electrospray ionization mass spectrometry (ESI- TOF), performed at the Multiuser Center for Biomolecules (CEMBio- RJ), enabled the comparative metabolomic profiling of samples collected from the five localities: Viana, Muniz Freire, Santa Teresa, Vitória (UFES campus), and Colatina. Data processing allowed the putative annotation or assignment of 20 known and 6 unknown secondary metabolites, as shown in Table 2. To facilitate understanding, these compounds were grouped into chemical classes according to the table below and related to their properties (Table 3).

Table 3
Main chemical classes and putatively annotated metabolites in *Tithonia diversifolia*.

Chemical category	Putatively annotated metabolites	Properties
Hydroxycinnamic acids and conjugated derivatives	Caffeoylquinic acid, Dicaffeoylquinic acid	Esters derived from the phenylpropanoid pathway; associated with antioxidant and metabolic activity.
Flavonoids	Naringenin, Apigenin, Hispidulin and Kaempferol dihexoside	Class widely related to antioxidant, anti-inflammatory, and bioactive activity.
Saturated and unsaturated fatty acids	Palmitic acid, Gamma-linolenic acid	Structural and bioactive lipid compounds, participate in metabolic pathways and physiological processes.
Sesquiterpene lactones	Tagitinin A	Characteristic metabolite of the Asteraceae family presents anti-inflammatory and cytotoxic properties.
Condensed tannins	Proanthocyanidin dimer	High molecular weight polyphenols, associated with antioxidant activity and complexation capacity.

Source: Own authorship.

The obtained chemical profile reveals that the plant material from the five localities exhibits a composition rich in secondary metabolites with synergistic potential for the antioxidant and antiproliferative activities reported in the literature (Zhang et al. 2019). As illustrated in Table 3, Tithonia diversifolia was characterized by the presence of hydroxycinnamic acids and their derivatives, flavonoids, saturated and unsaturated fatty acids, sesquiterpene lactones, and condensed tannins. Among these, hydroxycinnamic acids and their derivatives, represented by caffeoylquinic (**1**) and dicaffeoylquinic acids (**2**), and flavonoids, represented by naringenin (**3**), apigenin (4), and hispidulin (**5**), are recognized for their remarkable antioxidant properties and their potential to modulate cell proliferation, thereby contributing to the biological activity of the extracts (Pang et al. 2021). Recent studies have highlighted the role of flavonoids such as quercetin, genistein, and apigenin in inducing apoptosis in tumor cells, acting through modulation of Bax/Bcl-2 protein expression, caspase activation, and cytochrome c release (Kopustinskiene et al. 2020). These mechanisms reinforce the chemopreventive and antitumor potential attributed to the flavonoid compounds present in the extract analyzed here, suggesting that their antiproliferative activity may be associated with both antioxidant action and modulation of programmed cell death pathways. According to Ojo et al. (2018) this mechanism acts through direct neutralization of free radicals, chelation of pro-oxidant metals, and possible enzyme inhibition, a protection complemented by the sequestering action of the identified proanthocyanidin dimers, characteristic of the Asteraceae family (Rolnik and Olas 2021).



Thus, mass spectrometry analysis detected some point variations in certain compounds, such as the flavone apigenin, which was absent exclusively in the MUF5 sample. This pattern was similar to that observed for 6-methyl luteolin (absent in MUF5 and VIA5), suggesting a specific modulation of the biosynthetic pathway of methoxylated flavones in these samples. Another sample that showed a differentiated distribution profile was oxo-dihydroxy octadecenoic acid, which was absent in all three years of the UFES sample, while appearing irregularly in the other localities. The presence and variation in the levels of this oxygenated fatty acid suggest that it may belong to the class of oxidized lipid mediators, commonly known as oxylipins in plant physiology. These compounds are derived from enzymatic or non-enzymatic oxidation of polyunsaturated fatty acids, such as linolenic and linoleic acids (8), alongside primary lipophilic components like palmitic acid **(7)**, which together act as structural components and signaling molecules in responses to biotic and abiotic stresses (Taiz et al. 2017).

The quantitative variation among populations may reflect differences in the specific environmental conditions of each locality (such as light incidence, water availability, or pollutant presence) that induce distinct levels of oxidative stress. Thus, the fluctuations observed in this lipid mediator not only indicate a differentiated adaptive response of the Tithonia diversifolia populations to their microenvironments but also suggest variations in the bioactive potential of the extracts.

It is worth noting that isolated absences, such as that of caffeoylquinic acid in COL5 and the caffeoyl glycerol derivative in VIA4 and SANT5, point to natural intrapopulational variability. Overall, the data demonstrate that, although the studied species exhibits some prominent and robust compounds, the qualitative expression of specific metabolic pathways may be modulated by local factors, implying phenotypic plasticity in the plant and providing a solid foundation for future quantitative investigations and correlation studies with environmental factors.

Regarding antiproliferative action, apigenin and naringenin stand out for their biological relevance, described in this study as modulators of cell signaling pathways (PI3K/Akt, MAPK) and inducers of apoptosis (Stabrauskiene et al. 2022). Furthermore, the methoxylated flavone hispidulin and the kaempferol (6) glycoside exhibit selective cytotoxicity. In parallel, sesquiterpene lactones such as tagitinin A (9) and C, the latter reported by Ranti et al. (2018) as antifibrotic agents in keloid fibroblasts, also display potent cytotoxic activity, attributed to the α-methylene-γ-lactone group, which frequently acts via inhibition of NF-κB.

Additionally, the action of SLs is linked to their chemical structure, particularly the presence of the α-methylene-γ-lactone group, characterized by an unsaturated lactone ring, acting as a potent electrophile capable of alkylation reactions with cysteine residues in target proteins. As highlighted by Chadwick et al. (2013) this reactivity is the molecular basis for understanding the inhibition of the pro- inflammatory transcription factor NF-κB, either through direct modification of its p65 subunit or by interference with the IκB kinase complex, leading to cytotoxic and antiproliferative effects observed in cancer cell lines.Therefore, the spectrometric data not only validate the chemical identity of the species but also provide a solid chemical foundation for predicting and justifying future pharmacological assays aimed at exploring the chemopreventive and antitumor properties of this extract.

4 Conclusions

This study provided a comprehensive chemical characterization of hydroalcoholic extracts of Tithonia diversifolia by integrating FTIR spectroscopy, ESI-TOF mass spectrometry, and chemometric approaches. The results demonstrated that the chemical profile of the species varies according to geographical origin and phenological conditions, with Santa Teresa representing the most distinct and variable population among those evaluated. The combination of analytical techniques allowed the discrimination of metabolic patterns among populations and the putative annotation of secondary metabolites belonging to relevant chemical classes, supporting the biological potential previously described for this species. The evaluation of extract stability after 10 years of storage indicated that, although the overall chemical profile was preserved in most samples, specific populations exhibited greater metabolic variation over time, highlighting the importance of environmental and collection factors in the reproducibility of botanical extracts. Overall, this study contributes to a better understanding of the chemical variability and temporal stability of T. diversifolia extracts, providing relevant information for future pharmacological investigations and strategies aimed at the standardization of plant-derived products.

Declarations

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Author Contribution Statement

Ana Carolini Cavallieri Zatta was responsible for the conceptualization of the study, data curation, investigation, and preparation of the manuscript. Isadora Maria Coelho Vieira contributed to the translation of the manuscript and assisted in data interpretation. Lucas Evangelista dos Santos performed the multivariate statistical and chemometric analyses. Maria Gabriela Pissinati Trindade conducted the chemical analyses and experimental procedures. Mirieli Bernades Xavier contributed to the writing and organization of the manuscript Paula Roberta Costalonga Pereira contributed to the writing and organization of the manuscript. Maria do Carmo Pimentel Batitucci supervised the study, critically reviewed the manuscript, and approved the final version for publication.

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Tables

Table 2 is not available with this version.

Figures

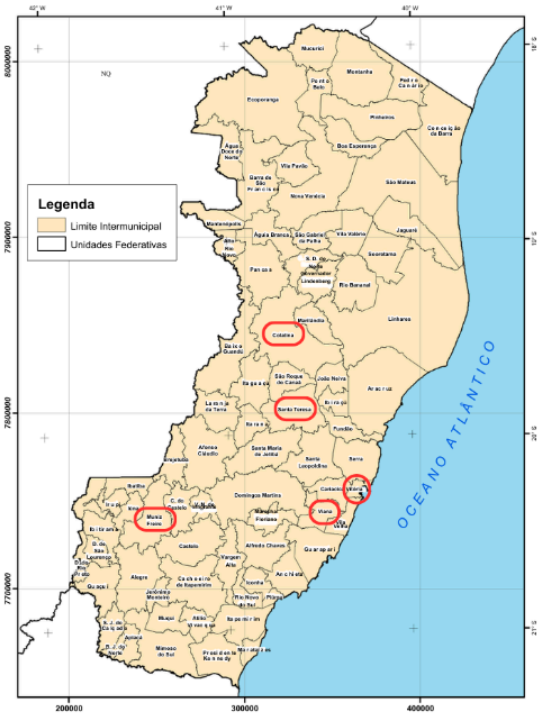

Figure 1

Geographic distribution of sampling sites in Espírito Santo State, Brazil, highlighting the municipalities where plant material was collected. Adapted from Geobases – Integrated System of Geospatial Databases of the State of Espírito Santo (2026).

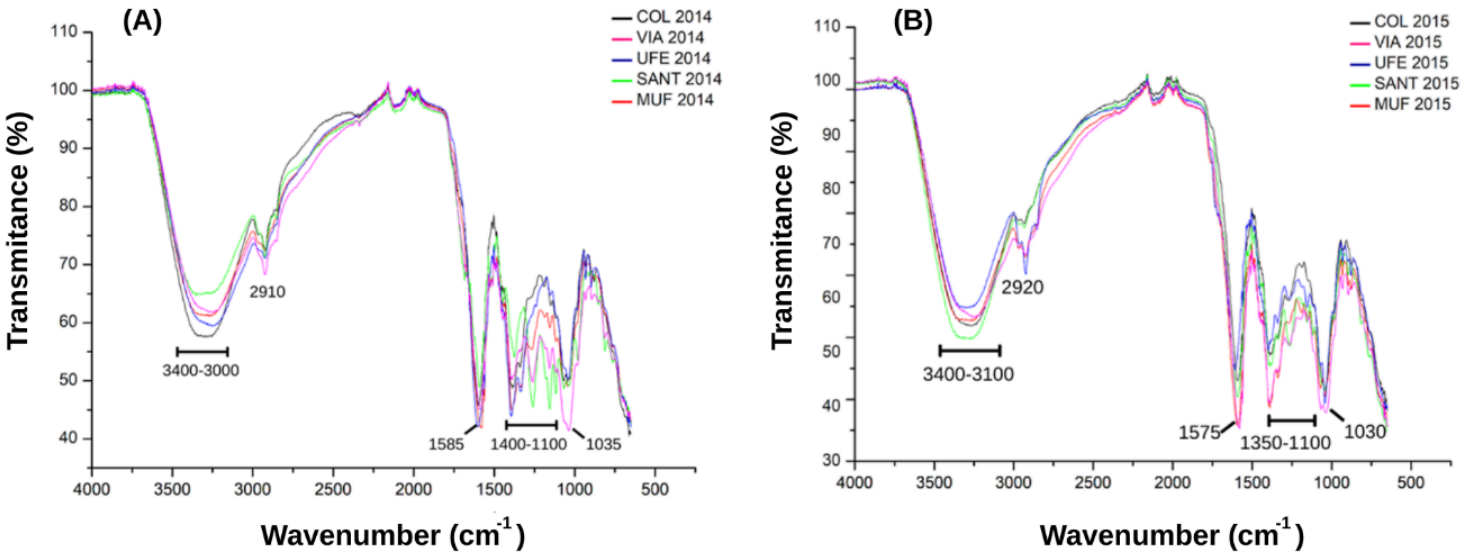

Figure 2

FTIR spectra of hydroalcoholic leaf extracts of *Tithonia diversifolia* from five localities in Espírito Santo. (A) FTIR spectra of extracts obtained in 2014; (B) FTIR spectra of extracts obtained in 2015. COL (Colatina); VIA (Viana); UFE (Ufes- Vitória), SANT (Santa Teresa); MUF (Muniz Freire).

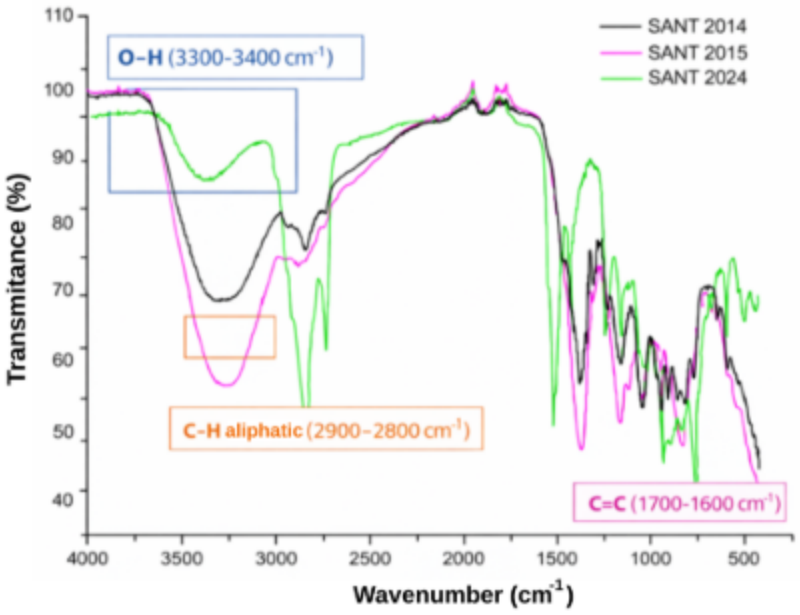

Figure 3

FTIR spectra of hydroalcoholic leaf extracts of *Tithonia diversifolia* from Santa Teresa

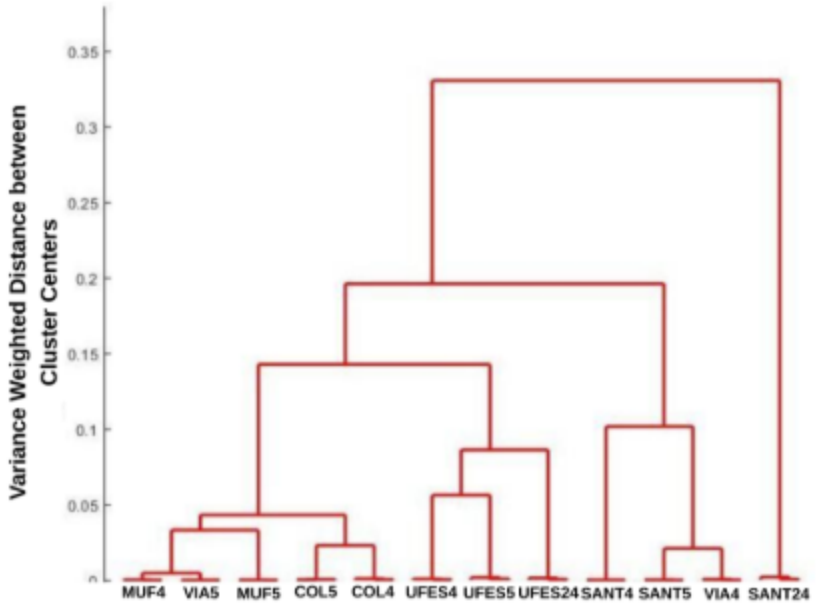

Figure 4

Hierarchical Cluster Analysis (HCA) of leaf extracts of *Tithonia diversifolia* from different localities and collection years.

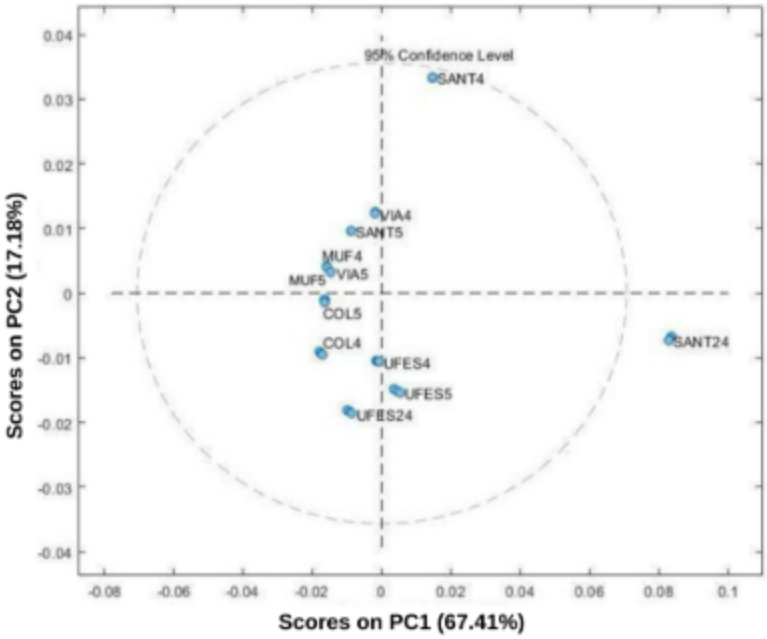

Figure 5

Principal Component Analysis (PCA) score plot of *Tithonia diversifolia* extracts from different geographical locations. PC1 and PC2 explained 67.41% and 17.18% of the total variance, respectively. The dashed circle indicates the 95% confidence level.

Supplementary Files

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