

Soil gradients and phytochemical responses in *Tithonia diversifolia*: design of a comprehensive utilization model by vegetative tissue in Veracruz

Gradientes edáficos y respuestas fitoquímicas en *Tithonia diversifolia*: diseño de un modelo de utilización integral por tejido vegetativo en Veracruz

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Classification:

Area: Engineering
Field: Chemical engineering
Discipline: Chemical Engineering
Subdiscipline: Bioengineering

doi <https://doi.org/10.35429/JMQM.2025.9.15.2.1.12>

History of the article:

Received: October 30, 2025

Accepted: December 30, 2025

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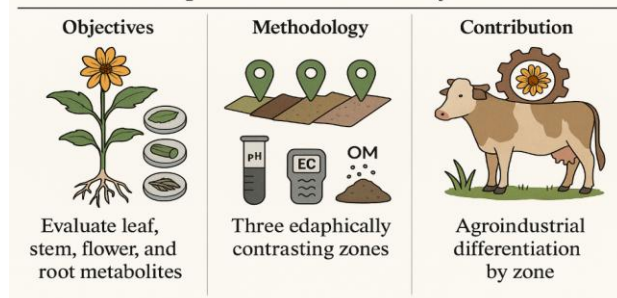
Abstract

Tithonia diversifolia [Hemsl.] A. Gray, an underutilized native plant, has industrial and antibiotic potential thanks to its richness in secondary metabolites and fatty acids. In this research, vegetative tissues [flower, leaf, stem and root] from three locations [Orizaba, Ixtaczoquitlán and Rafael Delgado, Veracruz], with different soil conditions were analyzed: Hydrogen Potential [pH], Organic Matter [OM], Electrical Conductivity [EC] and Texture [T], evidencing differentiated phytochemical profiles. High levels of palmitic acid [up to $26.80 \pm 16.72 \mu\text{g}$ in the flower] and stearic acid [up to $18.81 \pm 11.43 \mu\text{g}$] were noted, both with antimicrobial applications. The leaves have a high protein [$27.25 \pm 0.14\%$] content for livestock use, and the stem and root are used as soil improvers. This characterization highlights the value of the *T. diversifolia* plant genetic resource for its use in the agroindustrial, livestock, agricultural, and pharmaceutical sectors, promoting its revaluation through vegetative tissue, productive areas with a sustainable agroecological approach that can be incorporated into silvopastoral systems.

Resumen

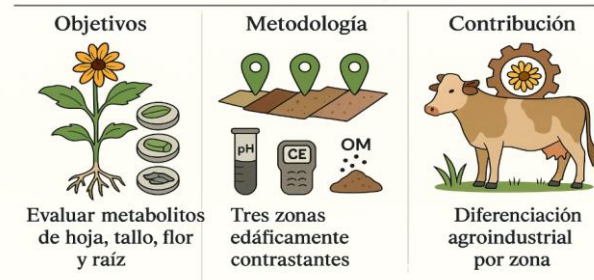
Tithonia diversifolia [Hemsl.] A. Gray, planta nativa subutilizada, posee un potencial industrial y antibiótico debido a su riqueza en metabolitos secundarios y ácidos grasos. En esta investigación, se analizaron los tejidos vegetativos [flor, hoja, tallo y raíz] provenientes de tres localidades [Orizaba, Ixtaczoquitlán y Rafael Delgado, Veracruz], con distintas condiciones edáficas: Potencial de Hidrógeno [pH], Materia Orgánica [MO], Conductividad Eléctrica [CE] y Textura [T], evidenciando perfiles fitoquímicos diferenciados. Destacaron contenidos elevados de ácido palmítico [hasta $26.80 \pm 16.72 \mu\text{g}$ en flor] y esteárico [hasta $18.81 \pm 11.43 \mu\text{g}$], ambos con aplicaciones, antimicrobianas, en hojas posee un alto contenido de proteínas [$27.25 \pm 0.14\%$] para uso pecuario, el uso de tallo y raíz, como mejoradores de suelos, esta caracterización resalta el valor del recurso fitogenético de *T. diversifolia* para su aprovechamiento en sectores: agroindustrial, pecuario, agrícola y farmacológico, promoviendo su revalorización por tejido vegetativo, área productiva con enfoque agroecológico sustentable que se incorpore a sistemas silvopastoriles.

Edaphic gradients and phytochemical responses in *Tithonia diversifolia*



Phytochemical profiling, edaphic gradients, *Tithonia diversifolia*

Gradientes edáficos y respuestas fitoquímicas en *Tithonia diversifolia*



Perfil fitoquímico, gradientes edáficos, *Tithonia diversifolia*

Area: Promotion of frontier research and basic science in all fields of knowledge

Citation: Ixmatlahua-Rodríguez, Christian Andrés, Ortiz-Celiseo, Araceli, Alejandro-Rosas, Jorge Alberto and López-Zamora, Leticia. [2025]. Soil gradients and phytochemical responses in *Tithonia diversifolia*: design of a comprehensive utilization model by vegetative tissue in Veracruz. Journal-Mathematical and Quantitative Methods. 9[15]1-12: e2915112.



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Introducción

Tithonia diversifolia [Hemsl.] A. Gray, commonly known as Mexican sunflower, is a herbaceous species native to Mexico that has been categorized by the National Commission for the Knowledge and Use of Biodiversity [Comisión Nacional para el Conocimiento y Uso de la Biodiversidad; CONABIO, 2009] as a rustic plant or even classified as a weed in various agricultural systems due to its rapid growth and high colonization capacity. However, recent research has revalued its agro-industrial and ecological potential, highlighting its adaptability to diverse soil types, rich phytochemical content, and outstanding bromatological properties.

As a forage species, *T. diversifolia* exhibits high biomass production and significant levels of crude protein, reaching up to 29% on a dry matter basis, positioning it as a strategic resource for sustainable silvopastoral systems [Uu-Espens *et al.*, 2021; Rivera *et al.*, 2021]. Additionally, its application in phytoremediation has been documented, demonstrating an efficient accumulation of heavy metals such as lead and zinc without compromising vegetative growth [Kekere *et al.*, 2020]. In the medicinal and ethnobotanical realm, *T. diversifolia* is widely used in rural communities for its anti-inflammatory, antioxidant, and antimicrobial properties, which are attributed to bioactive compounds including flavonoids, quinones, and phenolic acids [Monroy, 2025; Souza-Silva *et al.*, 2020].

Additionally, ecological modeling under climate change scenarios [2041–2080] has projected a slight expansion in the potential distribution of *T. diversifolia* in Mexico, with an estimated increase from 30.7% to 32.4% of the national territory, favored by its high tolerance to intense precipitation and elevated temperatures. This adaptive behavior suggests that its dispersal could be enhanced, consolidating its strategic role in resilient productive systems and in the restoration of degraded landscapes under a sustainable agroecological approach [Durán *et al.*, 2020; Pérez *et al.*, 2025].

Complementarily, physiological modeling studies such as Bayona [2023] demonstrate that thermal and radiation variations can significantly modulate the productivity and quality of agricultural species, highlighting the importance of analyzing the edaphoclimatic and metabolic response *T. diversifolia* under contrasting environmental scenarios.

This study aimed to analyze the presence of key secondary metabolites, moisture content, and fatty acid composition in four vegetative tissues [leaf, stem, flower, and root] of *T. diversifolia* from three wild populations across three locations in central Veracruz [Orizaba, Ixtaczoquitlán, and Rafael Delgado], considering their soil gradients. Variables such as pH, electrical conductivity [EC], organic matter, and soil texture directly influenced the production of secondary metabolites and nutritional parameters. It was observed that alkaline soils with high EC, as in Rafael Delgado, favored the accumulation of unsaturated compounds and fatty acids; whereas acidic to neutral soils in Ixtaczoquitlán and Orizaba produced lower amounts but considerable ash and protein contents, indicators of forage nutritional value. It was hypothesized that the edaphic environment modulates the agro-industrial use potential by tissue type, with differentiated applications in regional silvopastoral systems, antimicrobials, fertilizers, and soil improvement.

Materials and Methods

Georeferencing soil sampling

The study was conducted across three representative locations in the central region of the state of Veracruz, Mexico. The first site was situated in the community of Cuautlapan, municipality of Ixtaczoquitlán, at an altitude of 990 meters above sea level [masl] with geographic coordinates 18°52'23.91" N and 97°01'33.16" W. The second site was in Rincón Grande, municipality of Orizaba, located at 1,182 masl, with coordinates 18°52'45.12" N and 97°00'38.25" W. Lastly, the third sampling point was established in Jalapilla, municipality of Rafael Delgado, at an altitude of 1,177 masl, positioned at coordinates 18°49'25.57" N and 97°05'03.01" W [Google Earth, 2024].

These locations were selected for their stable wild populations of *T. diversifolia* occurring under contrasting agroecological conditions, enabling the assessment of edaphoclimatic influences on phytochemical composition and biomass utilization potential [CONABIO, 2009].

Soil samples were collected using a systematic transect design, randomly selecting 30 *T. diversifolia* plants per location. For each plant, soil adhering to the roots was extracted at an approximate depth of 20 cm.

Subsamples were homogenized to form a composite sample of 1 kg per locality, collected in duplicate [M1 and M2], and analyzed in triplicate according to NOM-021-RECNAT-2000 standards.

Soil analysis

Soil analyses were conducted at the Laboratory of the Faculty of Chemical Sciences, Veracruzana University [FCQ-UV], considering the following parameters: Organic Matter [OM] was measured using 10 g of soil, estimated by the Walkley-Black method modified by Kjeldahl. Soil texture was determined using the Bouyoucos method; 50 g of soil was dispersed in 100 mL of 0.5% sodium hexametaphosphate solution. Fraction readings were taken with a Bouyoucos hydrometer G.L.-5 at 60 VIRESA® at established intervals, allowing the quantification of sand, silt, and clay percentages.

For pH and Electrical Conductivity [EC], 20 g of soil were weighed and analyzed in soil:water [1:2] and soil:1N KCl [1:2] suspensions, continuously stirred for 30 minutes. Measurements were performed using a Hanna® Instruments H198130 multiparameter potentiometer [pH/EC/TDS, high range]. EC readings were obtained from the same solutions used for pH determination, to facilitate comparative analysis across acidic, neutral, and alkaline soils and their respective cation exchange capacities. All analyses followed the criteria established by Kome *et al.* [2018] and Quispe *et al.* [2019], adhering strictly to the guidelines of NOM-021-RECNAT-2000.

Sampling of plant tissues

A representative sampling area was delimited by selecting 30 uniform *T. diversifolia* plants per locality. The geographic location of each site was recorded for systematic documentation. Approximately 1,000 g of each vegetative tissue at physiological maturity, healthy and free of visible damage, were collected from each plant across the three localities. The vegetative material was washed with sterile distilled water to remove surface residues and subsequently air-dried by spreading each tissue sample evenly on clean mesh screens for 10 days in partial shade, at an average temperature of 22.88 ± 1.99 °C.

Moisture analysis

Moisture content determination was carried out at the Chemistry Laboratory of the Instituto Tecnológico de Orizaba [ITO], using previously dehydrated material from the vegetative tissues of *T. diversifolia*, following the methodology described by Montejo-Sierra *et al.* [2018]. Exactly 1.1 g of each sample was weighed and analyzed using an Ohaus® MB27 moisture analyzer. The procedure was performed in triplicate to ensure accuracy.

Ash and Protein Analysis.

Ash content was quantified by direct incineration in a muffle furnace, following the specifications of NMX-Y-362-SCFI-2019, which allowed estimation of total mineral content. Crude protein was estimated using the Kjeldahl method, according to NOM-F-68-S-1980, based on the determination of total nitrogen and its conversion to protein using the standard factor. All analyses were conducted in triplicate.

Secondary metabolites by High Performance Thin Layer Chromatography [HPTLC].

The chromatography was carried out in the Phytochemical Laboratory of the Center for Research and Advanced Studies of Irapuato [CINVESTAV, Unit Irapuato]. For the elution and visualization of metabolites, the High-Performance Thin-Layer Chromatography [HPTLC] technique was employed. Extracts diluted with 80% hydro-methanol were applied in aliquots of 10 µL onto silica gel 60 F254 plates with aluminum backing [250 µm thick, 10 × 20 cm, Sigma Aldrich].

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<https://doi.org/10.35429/JMQM.2025.9.15.2.1.12>

Applications were performed using a CAMAG Automatic TLC Sampler 4 at a rate of 10 $\mu\text{L}/\text{sec}$ and a bandwidth of 6.5 mm.

The software was programmed such that the first application was positioned 10 mm from the lower edge [X-axis] and 15 mm from the lateral edge [Y-axis].

The mobile phase consisted of a solvent system of ethyl acetate, formic acid, acetic acid, and water in the ratio 50:5.5:5.5:13 [v/v]. Before chromatographic development, the plates were dried for 30 seconds, followed by pretreatment of the chamber with the mobile phase at 20% relative humidity at room temperature. Subsequently, the plates were dried again for 5 minutes.

For the visualization of phytochemical compounds, a CAMAG TLC Visualizer [Switzerland] under white light was used, coupled with the visionCATS software version 2.0.15069.1, which enabled the digital capture of the chromatographic profiles [Wagner and Bladt, 2009].

Analysis by Gas Chromatography coupled with Mass Spectrometry [GC/EIMS]

Derivatization of extracts

Residual methanol in the extracts was removed by evaporation under a nitrogen stream within a fume hood. Subsequently, 20 μL of pyridine and 100 μL of N,O-bis[trimethylsilyl] trifluoroacetamide [BSTFA] were added as derivatizing agents. The samples were incubated for 30 minutes at 80 $^{\circ}\text{C}$ in a Thermomixer Comfort [Eppendorf®]. Finally, 100 μL of HPLC-grade isooctane was added to analysis vials to proceed with instrumental analysis. Each sample was processed in triplicate.

GC/EIMS Analytical Conditions

T. diversifolia samples were analyzed using gas chromatography coupled with electron impact ionization mass spectrometry [GC/EIMS]. An Agilent Technologies 7890A gas chromatograph coupled to a Hewlett Packard 5975C mass spectrometer was employed. Chromatographic separation was achieved using a DB-1MS column [J&W Scientific]. Ultra-high purity helium [99.9999%] served as the carrier gas at a constant flow rate of 1 mL/min.

ISSN: 2531-2979

RENIECYT: 1702902

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Derivatized samples [200 μL] were injected using an Agilent Technologies 7683B automatic injector at 150 $^{\circ}\text{C}$.

The oven temperature program started at 150 $^{\circ}\text{C}$ for 0.5 min, then increased at 12.5 $^{\circ}\text{C}/\text{min}$ to 310 $^{\circ}\text{C}$, held for 2.5 minutes. Perfluorotributylamine [PFTBA] was used as a mass calibration standard [Agilent Technologies], covering a detection range of 50 to 800 atomic mass units [amu]. Compound processing and identification were carried out using MSChem, NIST, and AMDIS software.

Quantification of fatty acids

A calibration curve was constructed on the gas chromatography-mass spectrometry system using linoleic acid standard [Sigma-Aldrich] at a known concentration [$\mu\text{g}/\mu\text{L}$]. The resulting polynomial equation was $y = 7 \times 10^9 X = 5 \times 10^7$, with a coefficient of determination $R^2=0.98$

This calibration curve was used to determine the concentrations of the different fatty acids present in the samples.

Statistical Analysis of Data

The chemical data derived from protein analysis [dry basis] and fatty acids obtained by GC/EIMS were subjected to statistical analysis using Minitab software version 18 [LLC, 2018].

Results

This study was conducted using a comparative approach between contrasting edaphological zones, with independent variables consisting of soil physicochemical parameters [see Table 1]: pH, electrical conductivity [EC], organic matter [OM], and texture [TS].

The dependent variables were moisture percentage, secondary metabolites, fatty acids, ash, and protein content, evaluated in four vegetative tissues [leaves, stems, flowers, and roots] *T. diversifolia* from three wild populations.

The pH values determined in the soils from the three localities [Orizaba, Ixtaczoquitlán, and Rafael Delgado] [Table 1] reflect significant edaphic gradients, consistent with previous findings where pH and electrical conductivity [EC] emerge as key factors in soil dynamics and microbiota.

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<https://doi.org/10.35429/JMQM.2025.9.15.2.1.12>

Box 1**Table 1**

Organic Matter, Texture, pH and Electrical Conductivity, content of soils from 3 locations in Veracruz.

Localidad	pH - H ₂ O		pH - KCl		Electrical Conductivity [EC]		O.M.	Soil texture
	M1	M2	M1	M2	M1- [dS/m]	M2- [dS/m]		
Orizaba	6.34 ± 0.20	6.27 ± 0.19	5.58 ± 0.12	5.54 ± 0.10	0.35 ± 0.10	0.27 ± 0.02	8.63 ± 0.10	Sandy Loam
Ixtaczoquitlán	7.07 ± 0.46	6.84 ± 0.41	6.22 ± 0.60	6.19 ± 0.61	0.47 ± 0.21	0.44 ± 0.23	2.72 ± 0.08	Sandy Clay Loam
Rafael Delgado	7.20 ± 0.11	7.22 ± 0.06	6.71 ± 0.12	6.75 ± 0.16	0.60 ± 0.29	1.21 ± 1.20	2.74 ± 0.06	Clay Loam

In this study, Rafael Delgado exhibited the highest pH values [7.20 ± 0.11] and EC [up to 1.21 ± 1.20 mS/cm], conditions that favor biochemical processes oriented towards the synthesis of secondary metabolites [Flores-Sánchez *et al.*, 2023], in agreement with reports for adaptive species in neutral-alkaline soils. Meanwhile, Ixtaczoquitlán, with intermediate pH [7.07 ± 0.46] and lower EC [0.47 ± 0.21], showed greater water retention, resulting in favorable stomatal moisture for growth and green biomass accumulation.

The low organic matter [OM] content in Ixtaczoquitlán [$2.72 \pm 0.08\%$] and Rafael Delgado [$2.74 \pm 0.06\%$] versus the highest value in Orizaba [$8.63 \pm 0.10\%$] and the differing soil textures [from sandy loam to clay loam] directly influenced the physical and chemical soil properties. An intermediate OM content combined with loam-clay texture balances good aeration and water storage, ideal for nutrient availability and water distribution fundamental agronomic principles.

These results indicate that soils with better structure and higher organic matter stabilize pH and EC, optimizing growth conditions and phytochemical expression *T. diversifolia* [Pant *et al.*, 2021]. Moisture content in the vegetative tissues [see Table 2] of *T. diversifolia* showed clear variation among localities, mainly influenced by edaphic factors such as soil pH, EC, and texture.

Box 2**Table 2** Percentage of moisture in *T. diversifolia* tissues

Tissues	Orizaba % Humidity	Ixtaczoquitlán % Humidity	Rafael Delgado % Humidity
Leaf	6.7 ± 0.52	7.0 ± 0.52	8.8 ± 0.52
Stem	7.9 ± 0.52	9.7 ± 0.52	9.7 ± 0.52
Root	7.6 ± 0.52	9.7 ± 0.52	9.7 ± 0.52
Flower	8.8 ± 0.52	9.7 ± 0.52	10.0 ± 0.00

Rafael Delgado exhibited the highest moisture values in nearly all tissues, particularly in the flower with 10%, which may be attributed to its greater water retention capacity linked to its loam-clay texture and higher electrical conductivity [1.21 ± 1.20 mS/cm]. This condition favors nutrient and water availability in the rhizosphere, facilitating water accumulation in vegetative structures [Holguín-Villanueva *et al.*, 2023]. The stems and roots from Ixtaczoquitlán and Rafael Delgado showed the same moisture content [$9.7 \pm 0.52\%$], indicating possible physiological adaptability of the plant to maintain water reserves in underground and supporting organs, especially in soils with intermediate texture and higher retention capacity. Orizaba, in contrast, exhibited the lowest moisture values across all tissues, with an average leaf moisture of $6.7 \pm 0.52\%$, consistent with its sandy loam texture, which limits soil water retention. These values suggest that the soil could be suitable for use as a soil conditioner or mulch [Li *et al.*, 2024].

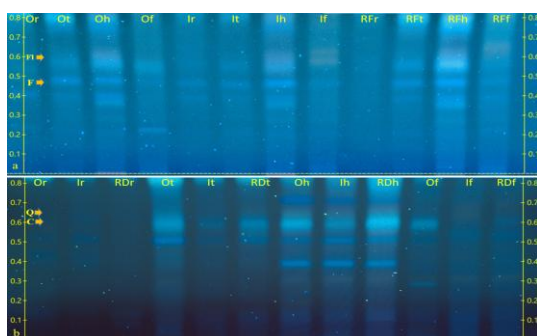
These results are important from a forage perspective, as moisture contents between 7–10% after drying guarantee good stability for silage, reducing the risk of undesirable fermentation and rot, thereby enhancing storage and use during scarcity periods. Furthermore, the flower and leaf tissues, with high moisture content, represent valuable fractions for formulating supplements rich in metabolites and with good digestibility [Ferrer *et al.*, 2021; Montoya-Flores *et al.*, 2022].

Ash values ranged between 6.36% and 15.23%, indicating significant variability in mineral accumulation based on sampling location [see Table 3]. The highest ash content in Ixtaczoquitlán [$15.23 \pm 0.02\%$] may be related to EC and nutrient retention capacity, which favors the absorption of essential minerals. Previous studies indicate that adaptive herbaceous species such as *T. diversifolia*, under fertile edaphic conditions, present higher mineral concentrations in tissues, translating to greater nutritional value for forage [Pant *et al.*, 2021]. A greater ash content denotes a higher amount of elements extracted from the soil, making it a good fertilizer or natural soil texture enhancer; apparent soil density increased significantly, as did moisture, porosity, and nutrient content [N, P, K, Ca, Mg], thereby improving crop growth and yield [Li *et al.*, 2020].

Box 3**Table 3**Ashes and Proteins in leaves of *T. diversifolia*

Análisis	Orizaba [%]	Ixtaczoquitlán [%]	R. Delgado [%]
Ashes	6.36 ± 0.23	15.23 ± 0.02	13.91 ± 0.22
Proteins [Dry Basis]	20.12 ± 0.03	25.56 ± 0.23	27.25 ± 0.14
Proteins [Wet Basis]	13.23 ± 0.13	16.19 ± 0.16	17.76 ± 0.07

The crude protein levels on a dry and wet basis also show differences among the populations. Ixtaczoquitlán [$25.56 \pm 0.23 / 16.19 \pm 0.16\%$] and Rafael Delgado [$27.25 \pm 0.14 / 17.76 \pm 0.07\%$] present higher values, supporting their use as high-quality forage feed. Rivera *et al.* [2021] report averages between 28–29% crude protein in *T. diversifolia*, especially in genotypes selected for similar altitudes. Furthermore, studies on the inclusion of this species in ovine diets demonstrated significant increases in crude protein intake without compromising digestibility or animal health [Adetola *et al.*, 2021], reaffirming its nutritional suitability according to the described edaphic variations. The chromatographic profiles in Figure 1 show the presence of four main types of metabolites in almost all samples: Flavonoids [F], Phenols [P], Quinones [Q], and Coumarins [C]. Bands corresponding to leaves [Oh, Ih, RDh] and flowers [Of, If, RDf] exhibit greater intensity in compounds of higher polarity [high Rf], suggesting a superior accumulation of flavonoids and phenols in stems [Ot, It, RDt] and roots [Or, Ir, RDr], especially in flowers from Orizaba and Rafael Delgado. Coumarins appear in flowers and leaves, and quinones in leaves [Oh, Ih, RDh] and stems [Ot, It, RDt], indicating adaptive responses induced by specific edaphic conditions.

Box 4

* Or, Ot, Oh, and Of refer to the root, stem, leaf, and flower tissues, respectively, from Orizaba. Similarly, Ir, It, Ih, and If denote the root, stem, leaf, and flower tissues from Ixtaczoquitlán. The abbreviations RDr, RDt, RDh, and RDf correspond to the root, stem, leaf, and flower tissues from Rafael Delgado.

Figure 1

Chromatographic profiles of four vegetative tissues from three wild populations of *T. diversifolia*

ISSN: 2531-2979

RENIECYT: 1702902

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High-Performance Thin-Layer Chromatography [HPTLC] enabled the identification of key metabolites *T. diversifolia*, highlighting flavonoids [F], phenols [P], quinones [Q], and coumarins [C], with well-defined bands reflecting their differential abundance across tissues and locations [see Table 4]. Notably, flowers and leaves from the three populations exhibited intense bands with retention factors [Rf] typical of more polar flavonoids and phenols.

Box 5**Table 4**Presence of secondary metabolites in plant tissues of *T. diversifolia*.

Metabolite	MeOH extract 80%			
	Flower	Leaves	Stems	Roots
Phenols	+	+	+	+
Flavonoids	+	+	+	-
Coumarins	+	+	-	-
Quinones	-	+	+	-

These molecules have high biological value due to their antioxidant, anti-inflammatory, and antibacterial activities, indicating considerable agro-industrial potential as sources of bioactives for phytotherapeutic formulations or natural biopesticides [Kaurinovic and Vastag, 2021; Tessema *et al.*, 2022]. Coumarins and quinones, associated with allelopathic and biostimulant properties, are valuable for incorporation into bioproducts aimed at soil improvement or as organic mulch for weed suppression.

This finding is consistent with the plant's adaptive behavior to soils with higher electrical conductivity and pH [as found in Rafael Delgado], where the production of these protective metabolites is intensified.

This tissue-specific pattern, reinforced by the visual resolution of the chromatographic profile, supports the design of a differential utilization model: leaves and flowers as sources for phytochemical inputs and natural antioxidants; roots and stems as bio-stimulant agents or soil enhancers.

Thus, the integral use of *T. diversifolia* aligns with sustainable valorization strategies in agroecological and silvopastoral systems aimed at organic agriculture, reducing production costs.

As shown in Table 5, the main fatty acids present in the vegetative parts of *Tithonia diversifolia* were identified and differentiated by locality. Table 6 presents the mass-based quantification, expressed as micrograms per gram of dry weight [$\mu\text{g/g DW}$], for the four analyzed vegetative tissues [leaf, stem, root, and flower] across the three study sites.

Box 6

Table 5

Main fatty acids present in the vegetative tissues of three populations of *T. diversifolia*

No.	Rt	Fatty acid	Vegetative organs from all localities	Use
1	39.665	C16:0 Palmitic Acid	Leaf, flower, stem and root	Antimicrobial Surfactant
2	43.152	C:18:0 Stearic Acid	Leaf, flower, stem and root	Antimicrobial Surfactant
3	42.535	C18:2n6 Linoleic Acid	Leaf, flower, stem and root	Antimicrobial, pharmaceutical, cosmetic, and food
4	42.62	C18:3n3 Alpha Linoleic Acid	Leaf	Antimicrobial, nutritional, and precursor to other compounds

Rt* Retention time

This quantification enabled an accurate comparison of the relative concentration of each fatty acid in the tissues, considering both the edaphic environment and the physiological role of the plant organ. Quantifying these compounds is essential, as fatty acids fulfill structural and defensive functions in plants, as well as possessing biotechnological potential as biostimulants, antimicrobials, or nutraceutical ingredients [Dongmo *et al.*, 2021]. This type of analysis contributes to assessing the differential utilization of each tissue according to its specific lipid composition.

Box 7

Table 6

Quantification in μg of important fatty acids present in plant tissues of *T. diversifolia*, in the three locations.

Plant Tissue	Fatty Acid	Orizaba Weight [$\mu\text{g/g DW}$]	Ixtaczoquitlán Weight [$\mu\text{g/g DW}$]	Rafael Delgado Weight [$\mu\text{g/g DW}$]
Flower	Palmitic Acid	13.76 \pm 0.63	16.06 \pm 1.84	26.80 \pm 16.72
Flower	Stearic Acid	10.22 \pm 0.60	7.27 \pm 3.48	18.81 \pm 11.43
Flower	Linoleic Acid	6.30 \pm 0.43	5.58 \pm 4.58	9.77 \pm 8.90
Leaf	Palmitic Acid	11.34 \pm 1.01	11.40 \pm 0.49	19.61 \pm 3.75
Leaf	Stearic Acid	8.12 \pm 0.54	8.66 \pm 0.50	12.99 \pm 2.01
Leaf	Linoleic Acid	3.55 \pm 0.24	3.02 \pm 0.07	7.28 \pm 1.48
Leaf	Alpha Linoleic Acid	4.86 \pm 0.42	3.83 \pm 0.06	9.72 \pm 1.97
Stem	Palmitic Acid	11.74 \pm 1.22	18.52 \pm 9.05	20.92 \pm 10.81
Stem	Stearic Acid	8.18 \pm 0.73	10.71 \pm 2.53	12.23 \pm 3.14
Stem	Linoleic Acid	2.75 \pm 0.16	3.06 \pm 1.25	2.91 \pm 1.18
Root	Palmitic Acid	10.99 \pm 6.63	14.21 \pm 0.57	18.31 \pm 6.99
Root	Stearic Acid	7.53 \pm 4.42	10.68 \pm 0.48	8.89 \pm 5.82
Root	Linoleic Acid	2.90 \pm 0.40	2.13 \pm 0.04	2.35 \pm 0.47

Gas chromatography coupled with mass spectrometry [GC-MS] phytochemical studies of plants like *Moringa oleifera* have extensively characterized fatty acid content, establishing benchmark reference parameters. High content is defined as concentrations above 15 $\mu\text{g/g}$ of dry tissue, especially in seeds, as reported by Gharsallah *et al.* [2022] and Cervera-Chiner *et al.* [2024], dominated by fatty acids such as palmitic, oleic, and stearic acids, associated with potential applications in biofuels and natural pharmaceuticals.

Moderate content [5–15 $\mu\text{g/g}$ dry weight] has been observed in vegetative structures like leaves or bulbs, where lipid profiles [Hosni *et al.*, 2022] fulfill structural or bioactive functions, in accordance with El-Naggar *et al.* [2023]. Low content [$<5 \mu\text{g/g}$ dry weight] is common in flowers or non-storage tissues, maintaining a lighter profile potentially involved in antioxidant or defensive activity. This classification guides biochemical interpretation of lipid content in non-conventional species like *T. diversifolia*.

Palmitic, stearic, linoleic, and α -linolenic acids were identified with tissue-specific profiles [flower, leaf, stem, root] and locality [Orizaba, Ixtaczoquitlán, Rafael Delgado].

Their biotechnological potential was assessed according to benchmark classification, enabling determination of whether a tissue serves as a rich, useful, or marginal source of specific fatty acids.

Flowers from Rafael Delgado showed high accumulation of palmitic acid [26.80 \pm 16.72 μg] and stearic acid [18.81 \pm 11.43 μg], while Orizaba and Ixtaczoquitlán maintained moderate levels. These long-chain saturated fatty acids are recognized for antimicrobial activity against Gram-positive and Gram-negative bacteria, targeting the cell membrane structure of pathogens. Their combination in petals, along with polyphenols and flavonoids per HPTLC analysis, positions them as natural biopesticides or ingredients for cosmetics and sanitizers [Kim *et al.*, 2021].

In Ixtaczoquitlán, palmitic [16.06 \pm 1.84 μg] and stearic [7.27 \pm 3.48 μg] levels were high-moderate, highlighting compositional consistency useful for standardized products.

Orizaba, with palmitic [$13.76 \pm 0.63 \mu\text{g}$] and stearic [$10.22 \pm 0.60 \mu\text{g}$], falls within the upper-moderate range, viable for general bio-inputs. Linoleic acid was moderate [$5.58 \pm 4.58 \mu\text{g}$] in all cases, useful as an anti-inflammatory or antioxidant.

Leaves of Rafael Delgado presented high palmitic acid [$19.61 \pm 3.75 \mu\text{g}$] and moderate α -linolenic acid [$9.72 \pm 1.97 \mu\text{g}$], suggesting protective or regulatory functions against oxidative stress, given that α -linolenic acid is an essential polyunsaturated fatty acid known to interfere with bacterial fatty acid synthesis by inhibiting key enzymes like FabI, explaining its antimicrobial action. Stearic acid [$12.99 \pm 2.01 \mu\text{g}$] was moderate, enhancing its utility in bioactive formulations, also attractive for cosmetic [moisturizing] and livestock supplement industries targeting ruminal microbiota modulation [Roopa *et al.*, 2020].

Palmitic acid concentrations were similar between Orizaba [$11.34 \pm 1.01 \mu\text{g}$] and Ixtaczoquitlán [$11.40 \pm 0.49 \mu\text{g}$], with stearic acid slightly predominant in Ixtaczoquitlán [$8.66 \pm 0.50 \mu\text{g}$] vs. Orizaba [$8.12 \pm 0.54 \mu\text{g}$]. α -Linolenic acid maintained stable moderate levels in both [$3.55 \pm 0.24 \mu\text{g}$ in Orizaba and $3.83 \pm 0.06 \mu\text{g}$ in Ixtaczoquitlán], indicating balanced potential for foliar bioprotectors or mild phytopharmaceutical agents useful in agroecological practices prioritizing sustainability and low toxicity. In stems, palmitic acid was high in Ixtaczoquitlán [$18.52 \pm 9.05 \mu\text{g}$] and Rafael Delgado [$20.92 \pm 10.81 \mu\text{g}$], matching benchmark criteria for a rich source with applications in silage, lipid supplementation, or structural mulch. Stearic acid [$10.71 \pm 2.53 \mu\text{g}$] was moderate, while linoleic acid remained low [$<3 \mu\text{g}$], indicating minor direct antioxidant role but synergistic antimicrobial potential as documented in other plant systems [Rouvier *et al.*, 2025]. Orizaba, though with lower values [palmitic $11.74 \pm 1.22 \mu\text{g}$], remained in the moderate range suitable for intermediate-use biomass. High palmitic acid content was determined in roots from Rafael Delgado [$18.31 \pm 6.99 \mu\text{g}$] and Ixtaczoquitlán [$14.21 \pm 0.57 \mu\text{g}$], suggesting lipid reserve accumulation with potential for biological control products, soil bioprotectors, or slow-release mulch systems, while Orizaba showed moderate level [$10.99 \pm 6.63 \mu\text{g}$], possibly related to denser or less porous soil characteristics. Stearic acid was moderate across all three sites.

Linoleic acid was low [$<3 \mu\text{g}$] in roots at all sites, limiting its biochemical prominence in this tissue but potentially participating as a cofactor in soil lipid synergies [Ferreira *et al.*, 2019].

Conclusions

The results obtained demonstrate that *T. diversifolia*, a species traditionally considered a weed, possesses highly differentiated agro-industrial and bioeconomic potential by tissue and locality, closely linked with edaphic gradients.

Edaphic analyses conclude that in Orizaba, leaves could be used for forage production, especially in systems requiring conservation by ensiling without additives. The stems and roots from Ixtaczoquitlán are recommended as mulch, green manure, or living cover due to their capacity to conserve moisture, suppress weeds, and enrich soil; stems also could complement forage because of their high protein content.

Phytochemical profile determination revealed that in Orizaba, flowers are rich in phenols and quinones, suggesting applications in essential oil and natural cosmetics industries. In Ixtaczoquitlán, leaves showed high flavonoid presence, with pharmacological and antioxidant implications. Finally, in Rafael Delgado, the phytochemical profile reflected phenols, flavonoids, quinones, and coumarins with potential antimicrobial, antibacterial, or natural sanitizing properties.

Differentiated fatty acid profiles were identified in *T. diversifolia*, highlighting palmitic, stearic, linoleic, and α -linolenic acids distributed specifically in flower, leaf, stem, and root tissues, modulated by local edaphoclimatic conditions. Fatty acid profile differentiation indicates that in Rafael Delgado, flowers exhibit the highest palmitic acid concentration, useful in bioherbicide or natural antipathogen formulations. Leaves showed elevated stearic and α -linolenic acids, important for phytopharmaceutical, cosmeceutical, and agricultural bio-stimulant formulations. Stems and roots in Ixtaczoquitlán and Rafael Delgado showed palmitic and stearic acids, suggesting possible use in biofertilizers, soil improvers, bioactive extracts, and lignocellulosic waste valorization with biological activity.

The distribution pattern also revealed Ixtaczoquitlán had the lowest standard deviation among replicates, suggesting greater metabolic stability in fatty acid synthesis, whereas Orizaba showed higher variability, especially in palmitic acid, possibly linked to microvariations in edaphic and management conditions, opening the possibility to adjust cultivation and harvest techniques for the desired metabolic product.

This integrative approach, based on accessible analytical technologies adapted to local conditions, allows designing viable technology transfer proposals for small producers, utilizing underused phylogenetic resources with low input requirements and high edaphic adaptability. The incorporation of *T. diversifolia* into silvopastoral production schemes, biofertilization, and compound extraction contributes to lowering production costs, promoting synthetic input substitution, and fostering a circular and sustainable agricultural economy in Veracruz.

The tissue- and locality-specific phytochemical-functional analysis highlighted how the edaphic conditions of each zone [Orizaba, Ixtaczoquitlán, and Rafael Delgado] differentially modulate this species' metabolic pathways, directly influencing the accumulation of secondary metabolites, fatty acids, and key phenolic compounds of functional and economic value.

Declarations

Conflict of interest

The authors declare that there is no conflict of interest. They maintain no financial, personal, or professional relationships that could influence the conduct or interpretation of this research.

They also do not receive incentives from private or commercial entities related to the use of *T. diversifolia*.

Author Contributions

Ixmattlahua-Rodríguez, Christian Andrés: Conceptualization of the project, methodological design, field sampling, compound analysis, and manuscript writing.

Ortiz-Celiseo, Araceli: Phytochemical analysis in the laboratory, table preparation, results interpretation, and technical discussion.

ISSN: 2531-2979

RENIECYT: 1702902

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Alejandro-Rosas, Jorge Alberto: Processing of edaphic data, literature integration, and introduction writing.

López-Zamora, Leticia: Institutional coordination, validation of results, scientific correction of the text, conclusions, and final editing of the document.

Data Availability

All data generated or analyzed during this study are available upon request from the reader, including raw matrices, pigment tables, chromatograms, and edaphic results. No commercial analysis platforms were used.

Funding

This project was funded by the scholarship number 2023-000002-01NACF-03692 awarded by the Secretaría de Ciencia, Humanidades, Tecnología e Innovación [SECIHTI], under the graduate studies support program.

Acknowledgments

We thank the technical support from soil and bioactive compound analysis laboratories of the participating institutions, as well as the collaboration of local producers for access to sampling sites.

Abbreviation

CE	Electrical Conductivity
HPTLC	High-Performance Thin-Layer Chromatography
MO	Organic Matter
Rf	Retention Factor
<i>T. diversifolia</i>	<i>Tithonia diversifolia</i>
UV	Ultraviolet

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