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Chemical characterization of the essential oil from the leaves of *Tithonia diversifolia* (Hemsl.) A. Gray: A combined GC/MS and ^{13}C NMR approach

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Abstract

The essential oil from the leaves of *Tithonia diversifolia*, collected in Daloa (Côte d'Ivoire), was extracted by hydrodistillation and analyzed using gas chromatography coupled with mass spectrometry (GC/MS) and carbon-13 nuclear magnetic resonance (^{13}C NMR). The extraction yield reached 0.044 ± 0.001%. Fifty-one compounds, accounting for 99.58% of the total oil, were identified and classified into seven chemical groups dominated by hydrocarbon monoterpenes (71.27%), followed by hydrocarbon sesquiterpenes (17.10%) and oxygenated sesquiterpenes (4.00%). The major constituents were sabinene (39.25%), α-pinene (29.66%), β-caryophyllene (8.61%), caryophyllene oxide (3.04%), α-farnesene (3.03%), a farnesene isomer (2.96%), phytol (2.36%), and α-cedrene (1.12%). The combined GC/MS–^{13}C NMR approach enabled the confirmation of the structures of the main constituents and the distinction of certain isomers that could not be differentiated by mass spectrometry alone. These results reveal a chemotype rich in hydrocarbon monoterpenes, suggesting potential biological activities, notably antimicrobial, antioxidant, and insecticidal. The study, through the complementary use of two analytical techniques, enhances the chemical knowledge of *T. diversifolia* and opens perspectives for valorization in pharmacy, agriculture, and cosmetics.

Keywords: *Tithonia diversifolia*; Essential oil; GC/MS; ^{13}C NMR; Chemical composition; Monoterpenes; Côte d'Ivoire

1. Introduction

Essential oils constitute an important reservoir of specialized metabolites, mainly monoterpenes and sesquiterpenes, whose biological and pharmacological properties are well documented (Bakkali et al., 2008). These substances are exploited both in traditional medicine and in the pharmaceutical, cosmetic, and agrochemical industries for their antimicrobial, antifungal, antioxidant, and insecticidal activities (Sharifi-Rad et al., 2017).

Tithonia diversifolia, commonly known as the Mexican sunflower, is a plant belonging to the Asteraceae family, widely distributed in tropical and subtropical regions (CABI, 2025). Native to Central America and naturalized in Africa, this species has attracted growing interest because of its medicinal and agroecological applications (Olayinka &

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Akinmoladun, 2019). It is characterized by rapid growth and a strong ability to adapt to various soil types and climatic conditions, making it a pioneer plant in many disturbed ecosystems (Sampaio et al., 2016).

Morphologically, T. diversifolia is a herbaceous or shrubby plant that can reach a height of 2–3 m. It has erect stems that are woody at the base, alternate leaves generally lobed (3 to 7 lobes) and covered with fine hairs, and bright yellow flowers grouped into capitula, typical of the Asteraceae family (Gualberto et al., 2011; Heuzé et al., 2016). Reproduction occurs mainly by seeds (achenes), dispersed by wind or animals, but also through cuttings (Olabode et al., 2007).

The leaves are used in traditional medicine for the treatment of various ailments such as malaria, pain, inflammation, and microbial infections (Akinmoladun et al., 2020). Several phytochemical studies have demonstrated that this species is rich in terpenoid and flavonoid compounds; however, data on the detailed composition of its essential oil remain limited and sometimes contradictory depending on the geographical origin (Ngassoum et al., 2004; Oyedepo et al., 2021).

The present study aims to characterize the essential oil of T. diversifolia leaves collected in Daloa (Côte d'Ivoire) by combining two complementary analytical techniques, GC/MS and ^{13}C NMR. This approach will not only establish the chemical profile of the essential oil but also confirm and refine the structural identification of its constituents by distinguishing isomers that are difficult to discriminate using a single technique.

2. Materials and Methods

2.1. Plant Material

The plant material consisted of the leaves of Tithonia diversifolia. The leaves were collected from the Daloa region (Côte d'Ivoire). After harvesting, they were stored at room temperature for 24 hours before being used for essential oil extraction.

2.2. Extraction of the Essential Oil (EO) by Hydrodistillation

Approximately 1.5 L of water was introduced into the distillation apparatus (cooker). A measured quantity of leaves was weighed and placed inside the cooker, which was then sealed and brought to a boil. The extraction lasted for three hours, starting from the appearance of the first drop of essential oil. This operation was repeated several times. The extracted essential oils were weighed, stored in amber glass vials, and kept in a freezer at a temperature close to 0 °C.

The extraction yield (Rdt) for each essential oil sample was calculated using the following formula:

$$\text{Rdt (\%)} = \frac{\text{Mass of EO obtained (g)}}{\text{Mass of dried plant material (g)}} \times 100$$

2.3. Characterization by GC/MS and ^{13}C NMR

The analysis of the essential oil extracts was performed using the following analytical techniques: carbon-13 nuclear magnetic resonance (^{13}C NMR) and gas chromatography coupled with mass spectrometry (GC/MS). The chromatogram of the essential oil, as well as the mass and ^{13}C NMR spectra of each compound, were recorded using a Bruker spectrometer (Bruker BioSpin AG). The elution solvent was deuterated chloroform ($CDCl_3$), and chemical shifts (δ, in ppm) were referenced to tetramethylsilane (TMS) as the internal standard.

The instrument was equipped with a 5–10 mm probe operating at 100.623 MHz for carbon-13. The ^{13}C spectra were recorded under the following conditions: 5 mm probe, 45° pulse angle, acquisition time = 2.73 s corresponding to a 64 K acquisition with a spectral width (SW) of 25,000 Hz (250 ppm), and a digital resolution of 0.183 Hz/pt. Approximately 70 mg of essential oil were dissolved in 5 mL of $CDCl_3$. The number of accumulations ranged from 2,000 to 5,000 for each run. Decoupling was carried out using the "Composite Phase Decoupling" pulse field method. The free induction decay (FID) signals were multiplied by an exponential function (LB = 1.0 Hz) before Fourier transformation.

3. Results

3.1. Extraction Yield

The extraction yield of essential oil obtained from the leaves of Tithonia diversifolia collected in Daloa was 0.044 ± 0.001%.

3.2. Combined GC–MS and ^{13}C NMR Analysis of the Constituents of Tithonia diversifolia Leaf Essential Oil

The chromatogram shown in Figure 1 corresponds to the gas chromatography–mass spectrometry (GC/MS) analysis of the essential oil extracted from the leaves of Tithonia diversifolia. This chromatographic profile reveals the presence of numerous peaks, some of which are well resolved, reflecting the chemical complexity of the essential oil. Each peak represents a volatile compound separated according to its retention time (Rt) and detector response (signal intensity). A high concentration of peaks is observed between 3 and 13 minutes, indicating the predominance of light and volatile constituents.

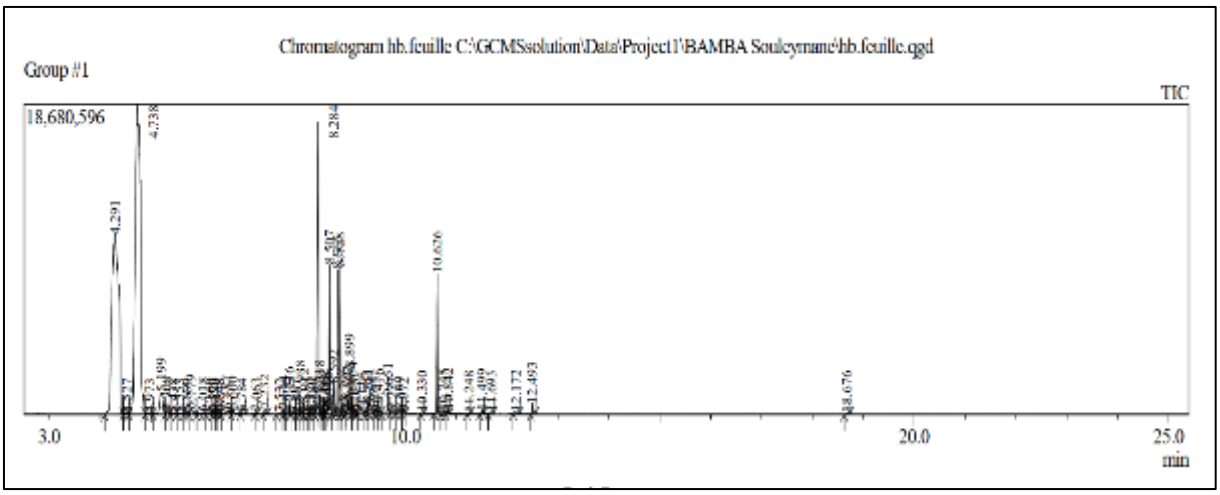

Figure 1 GC/MS chromatogram of the essential oil from Tithonia diversifolia leaves

Table 1 presents the chemical composition of the major compounds identified by GC/MS in the essential oil of Tithonia diversifolia leaves, with their relative percentages and corresponding ^{13}C NMR chemical shifts ($CDCl_3$, 100 MHz, δ in ppm). The main constituents are sabinene (39.25%), α-pinene (29.66%), β-caryophyllene (8.61%), caryophyllene oxide (3.04%), α-farnesene (3.03%), β-farnesene (2.96%), phytol (2.36%), and α-cedrene (1.12%).

Table 1 Major constituents of the essential oil from Tithonia diversifolia leaves: relative percentages and ^{13}C NMR chemical shifts ($CDCl_3$, 100 MHz), identified by GC–MS.

Compound	Relative %	^{13}C NMR ($CDCl_3$, 100 MHz, δ ppm)	Remarks
Sabinene	39.25	149.6 (Cq C=C), 111.2 (CH_2=), 50.4, 48.9, 38.2, 32.1, 29.5, 27.3, 23.8, 21.6, 18.4	Bicyclic monoterpene
α-Pinene	29.66	150.1 (Cq C=C), 109.6 (exocyclic CH_2), 50.3, 48.1, 43.9, 38.5, 34.2, 29.7, 27.1, 21.4, 16.1	Bicyclic monoterpene
β-Caryophyllene	8.61	148.6 (Cq C=C), 135.2 (Cq/CH=), 124.5 (CH=), 41.6, 39.8, 36.2, 33.9, 29.8, 28.2, 26.1, 23.7, 21.6, 16.2	Bicyclic sesquiterpene
Caryophyllene oxide	3.04	148.3 (Cq C=C), 124.1 (CH=), 62.3, 60.6 (oxirane carbons), 45.3, 43.9, 40.8, 36.1, 33.0, 30.1, 28.5, 25.7, 23.4, 17.2	Epoxidized sesquiterpene
α-Farnesene	3.03	147.5 (isopropenyl Cq), 139.1 (Cq), 136.8 (Cq), 125.6 (CH=), 124.3 (CH=), 110.7 (CH_2=), 39.8, 32.7, 26.8, 17.6, 16.2	Acyclic sesquiterpene
Farnesene (isomer)	2.96	147.2, 138.5, 134.2 (olefinic Cq), 125.1, 124.0 (CH=), 111.0 (CH_2=), 39.6, 32.5, 26.7, 17.6, 16.1	Profile similar to α-farnesene
Phytol	2.36	59.6 (CH_2–OH), 143.5 (Cq C=C), 123.7 (CH=), 39.8, 37.5, 32.9, 31.6, 29.8, 29.6, 28.9, 25.7, 22.7, 16.1 (terminal CH_3)	Diterpenoid (alcohol)
α-Cedrene	1.12	149.3 (Cq C=C), 122.1 (CH=), 48.7, 44.1, 39.6, 34.8, 30.3, 28.7, 26.2, 23.8, 21.2, 19.5, 16.3	Tricyclic sesquiterpene

The main constituents identified by GC/MS and ^{13}C NMR are shown in Figure 2**.** These include sabinene (1)**,** α-pinene (2)**,** β-caryophyllene (3)**,** caryophyllene oxide (4)**,** α-farnesene (5)**,** phytol (6)**,** β-farnesene (7)**,** and α-cedrene (8)**.**

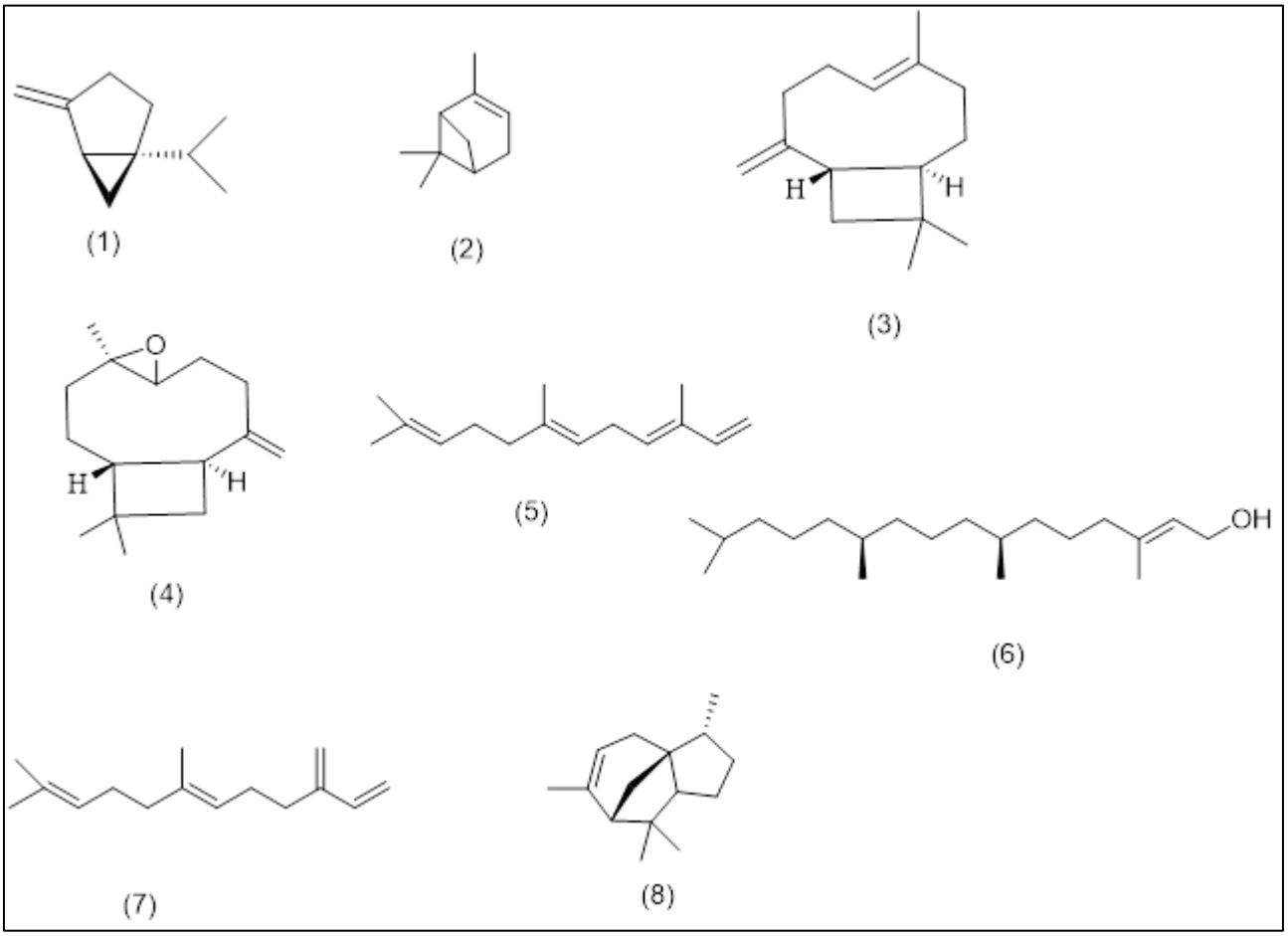

Figure 2 Chemical structures of the major compounds identified in the essential oil from Tithonia diversifolia leaves.

In addition to the major constituents, several other compounds present in relatively low proportions were also identified in the essential oil of Tithonia diversifolia leaves through gas chromatography–mass spectrometry (GC/MS) and carbon-13 nuclear magnetic resonance (^{13}C NMR) analyses.

Table 1 presents all the detected compounds, along with their retention times and relative percentages.

Table 2 Chemical composition of the essential oil from Tithonia diversifolia leaves.

Peak No.	Retention time (min)	Content %	Compound name
1	4.291	29.66	α-Pinene
2	4.738	39.25	Sabinene
3	4.973	0.17	Terpinene
4	5.308	0.21	Myrcene
5	5.438	0.08	*p*-Cymene
6	5.553	0.06	*m*-Cresol
7	5.699	0.11	Limonene
8	5.779	0.35	Linalool

9	6.018	0.14	Ocimene
10	6.160	0.05	Camphenone
11	6.229	0.04	Isophorone
12	6.280	0.02	*trans*-2-Nonenal
13	6.348	0.04	Pinocarvone
14	6.487	0.28	Terpinen-4-ol
15	6.600	0.23	α-Terpineol
16	6.784	0.04	Citronellal
17	7.063	0.04	Substituted trimethylbenzene
18	7.232	0.11	*p*-Hydroxyacetophenone
19	7.532	0.04	Myrtenyl acetate
20	7.614	0.06	Germacrene A
21	7.716	0.24	α-Cubebene
22	7.807	0.04	Indanone derivative
23	7.867	0.02	Geranyl acetate
24	7.938	0.47	Copaene
25	8.012	0.23	Germacrene D
26	8.180	0.04	Ylangene
27	8.284	8.61	β-Caryophyllene
28	8.318	0.22	α-Humulene
29	8.382	0.20	Farnesene
30	8.414	0.11	Germacrene B
31	8.466	0.05	Hydrogenated naphthalene
32	8.507	3.04	Caryophyllene oxide
33	8.592	0.73	Jasmonic acid derivative
34	8.665	2.96	Farnesene
35	8.725	3.03	α-Farnesene
36	8.796	0.36	β-Bisabolene
37	8.862	0.17	β-Hydrogenated naphthalene
38	8.899	1.12	α-Cedrene
39	8.964	0.29	Di-epi-α-Cedrene
40	9.025	0.03	Calacorene
41	9.114	0.33	Geraniol
42	9.251	0.28	Diethyl phthalate
43	9.476	0.21	Citral
44	9.651	0.37	Lidocaine
45	9.727	0.12	Camphorone
46	9.869	0.16	Cubenol

47	9.972	0.03	Heptatriacontanol
48	10.330	0.02	Nerolidol
49	10.626	2.36	Phytol
50	11.248	0.01	Ascorbic acid dihexadecanoate
51	11.499	0.13	Falcarinol

The classification of the constituents identified in the essential oil from Tithonia diversifolia leaves, presented in Table 3, reveals a clear predominance of hydrocarbon monoterpenes (71.27%), mainly represented by sabinene and α-pinene. Hydrocarbon sesquiterpenes (17.10%) constitute the second most abundant chemical class, followed successively by oxygenated sesquiterpenes and related derivatives (4.00%), long-chain alcohols and esters / diterpenes (2.80%), oxygenated monoterpenes (2.00%), aliphatic compounds and other derivatives (1.80%), and finally aromatic derivatives (0.65%).

Table 3 Distribution of the compounds identified in the essential oil from Tithonia diversifolia leaves according to their chemical classes.

Chemical class	**Compounds**	**Content %**
Hydrocarbon monoterpenes	α-Pinene, Sabinene, Terpinene, Myrcene, Limonene, Ocimene	71.27
Oxygenated monoterpenes	Linalool, Terpinen-4-ol, α-Terpineol, Citronellal, Camphenone, Pinocarvone, Citral, Geraniol, Geranyl acetate, Myrtenyl acetate	2.00
Hydrocarbon sesquiterpenes	Germacrene A, Germacrene D, Germacrene B, α-Cubebene, Copaene, Ylangene, β-Caryophyllene, α-Humulene, Farnesene, α-Farnesene, β-Farnesene, β-Bisabolene, α-Cedrene, Di-epi-α-Cedrene	17.10
Oxygenated sesquiterpenes and derivatives	Caryophyllene oxide, Cubenol, Calacorene, Nerolidol	4.00
Aromatic derivatives	*p*-Cymene, Substituted trimethylbenzene, *m*-Cresol, *p*-Hydroxyacetophenone	0.65
Aliphatic compounds and other derivatives	Isophorone, *trans*-2-Nonenal, Jasmonic acid derivative, Diethyl phthalate, Lidocaine, Heptatriacontanol, Ascorbic acid dihexadecanoate	1.80
Long-chain alcohols and esters / Diterpenes	Phytol, Falcarinol	2.80

4. Discussion

The essential oil of Tithonia diversifolia leaves collected in Daloa showed a yield of 0.044 ± 0.001%, a relatively low value but comparable to those reported for leaf essential oils of the same species (Ngassoum et al., 2004). The chemical composition is dominated by hydrocarbon monoterpenes (71.27%), mainly sabinene (39.25%) and α-pinene (29.66%), followed by hydrocarbon sesquiterpenes (17.10%), including β-caryophyllene (8.61%) and α-farnesene (3.03%). Oxygenated sesquiterpenes (caryophyllene oxide, 3.04%), diterpenes (phytol, 2.36%), and other aromatic and aliphatic compounds constitute minor fractions. This distribution reveals a sabinene/α-pinene chemotype, distinguishing the Daloa oil from those reported elsewhere.

The combined use of GC/MS and ^{13}C NMR allowed for a more reliable identification of the constituents. The NMR analysis confirmed the presence of farnesene isomers and oxygenated derivatives that are often difficult to separate using mass spectrometry alone, thereby demonstrating the relevance of an integrative analytical approach (El Hafidi et al., 2023).

The chemical profiles of T. diversifolia essential oils vary considerably according to geographical origin. In Latin America, chemotypes dominated by tagetone and β-ocimene have been reported (Goffin et al., 2002). In East and West Africa, the profiles are more diverse, ranging from oils rich in oxygenated sesquiterpenes (Ngassoum et al., 2004) to those dominated by germacrene D or caryophyllene (Oyedepo et al., 2021). The composition observed in Daloa, marked by the abundance of sabinene and α-pinene, confirms the existence of geographically distinct chemotypes. Such differences can be attributed to ecological factors (soil type, climate, altitude) and harvest conditions (phenological stage, seasonality).

Compared to other species of the genus Tithonia, particularly Tithonia rotundifolia, the essential oil of T. diversifolia shows a higher proportion of hydrocarbon monoterpenes, suggesting a species-specific metabolic profile. Indeed, the essential oil of T. rotundifolia is mainly composed of sesquiterpene hydrocarbons, which represent about 78.1% of its total composition, with germacrene D (33%) and β-caryophyllene (25.8%) as the main components (Gbolade, 2008).

Chemical variation in essential oils is often linked to environmental conditions and the metabolic plasticity of plants. In Daloa, the humid tropical climate**,** ferrallitic soil, and harvest period may explain the high proportion of monoterpenes. The hydrodistillation method used here may also influence the profile obtained, as it favors the extraction of thermoresistant volatile compounds while limiting the recovery of more labile molecules (Elyemni et al., 2019).

One of the original aspects of this work lies in the use of ^{13}C NMR to confirm and complement the GC/MS data. This technique made it possible to resolve ambiguities concerning certain isomers, particularly farnesene derivatives, and to detect signals corresponding to minor compounds not referenced in conventional spectral databases. The combined analytical approach thus enhances the reliability and robustness of structural identification.

The composition obtained suggests notable biological properties**.** Sabinene and α-pinene are known for their antimicrobial, antioxidant, and insecticidal activities (Silva et al., 2012). β-Caryophyllene and its oxide are widely studied for their anti-inflammatory and analgesic properties (Fidyt et al., 2016). The presence of phytol, a diterpene with antioxidant and cytoprotective activities, also adds pharmacological interest (de Morais et al., 2014). Therefore, the essential oil of T. diversifolia could be exploited in medicinal and agrochemical applications, particularly as a biopesticide or natural repellent.

To our knowledge, this is the first study combining GC/MS and ^{13}C NMR for the characterization of T. diversifolia essential oil in Côte d'Ivoire. It highlights a unique chemotype dominated by sabinene and α-pinene, thereby enriching the understanding of intraspecific chemical variability within this species. The results obtained open up promising perspectives for the valorization of T. diversifolia as a source of bioactive natural compounds of interest for traditional medicine, sustainable agriculture, and the cosmetic and food industries.

5. Conclusion

The study conducted on the essential oil from the leaves of Tithonia diversifolia collected in Daloa (Côte d'Ivoire) revealed a chemical composition dominated by hydrocarbon monoterpenes, with sabinene and α-pinene as the major constituents. The combination of GC/MS and ^{13}C NMR analytical techniques allowed for accurate structural identification, confirming the existence of a distinct chemotype compared to those reported in other regions. These findings, in addition to their chemosystematic significance, suggest promising biological and economic valorization potential for the essential oil of T. diversifolia.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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