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Research Article

Chemical Investigation on Essential Oil Composition of *Tithonia diversifolia* Growing Wild in Côte d'Ivoire

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Abstract

Tithonia diversifolia is widely used in African and American traditional medicine. Several biological and pharmacological studies have been carried out, using extract and essential oil of *Tithonia diversifolia*. Considering the numerous medecinal properties that justify the interest of continuing the chemical description of essential oil from this species. The present study aims to investigate essential oils from fresh organs (leaves, flowers, stems, roots) of *Tithonia diversifolia,* growing in four localities of Côte d'Ivoire. The essential oils are extracted by hydrodistillation using a Clevenger-type apparatus. 67 compounds were obtained by GC-MS analysis, with the most dominant being monoterpene hydrocarbons, followed by sesquiterpene hydrocarbons, oxygenated monoterpenes and oxygenated sesquiterpenes. The description of the essential oil (EO) composition from *Tithonia diversifolia's* roots shown α-pinene (95.05 to 97.01%), modephene (14.59 to 15.77%), *β*-pinene (1.46 to 10.05%) and *α*-isocomene (7.03 to 8.43%) as major compounds. Leaves essential oil was characterized by a predominance of *α*-pinene (8.66 to 29.76%), limonene (8.43 to 49.02%) and *trans-β*-ocimene (18.05 to 28.35%). In addition, stem oil was dominated by *α*-pinene (68.4 to 88.03%), allowed by *β*-pinene (2.78 to 15.05%) and limonene (7.39 to 14.29%). Limonen (33.08 to 40.07) was a main compound for flowers oil. In front of the chemical variability of the EO compositions, the results of analysis were submitted to hierarchical cluster. Two groups G1 and G2 were found, dominated by *α*-pinene and *cis* bisabolen respectively

Keywords

Tithonia diversifolia, Essential Oil Composition Roots, Pinene, Hierarchical Cluster

1. Introduction

The genus Tithonia comprises over 13 species in the Asteraceae family, includes herbaceous plants, annuals, invasive perennials and perennial shrubs [1]. This genus is found in several regions, including southern Mexico, Guatemala, Panama, Honduras, Cuba, Venezuela and Colombia [1]. When *Tithonia diversifolia* (Hemsley) A. Gray,

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pan-tropical plant species, hold a significant place in traditional medicine. Antioxidant [2], anti-inflammatory [3], antidiarrheal [4], antiplasmodial [5, 6] and antihyperglycemic properties [7] have been reported. Its use as an effective fertilizer for soil amendment was studied [8].

In regards to the phytochemistry review of *Tithonia diversifolia*, a wide variety compounds have been identified. Its extracts were the rich source of flavonoids and sesquiterpenes lactone [9]. Moreover, alkaloids, tannins, saponins, steroids, glycosides, terpenoids, proteins and phenols were presents [10].

Concerning essential oils from *Tithonia diversifolia*, several papers reported the most important components as limonene (20.1%), *α*-copaene (10.3%), o-cymene (10%), isocaryophyllene (8.73%), *α*-pinene (7.72%; 18.97%), 1.8-cineole (14.6%) from Cameroun [11, 12]; *α*-pinene (63.64%), *β*-pinene (15%) and isocaryophyllene (7.62%) from Kenya [13]; piperitone (11.72%), *α*-pinene (9.9%), limonene (5.40%), (Z)-β-ocimene (4.02%), p-cymen-8-ol (3.0%), (E)-nerolidol (3.78%) and spathulenol (10.8%) [14] from Brazil; α-pinene (63.62%), bicyclo [3.1.0] hexane 4-methylene-1-(1-méthyl éthyl)- (14.68%) from India [15].

Our previous study dealing with aerial parts (leaf, stem) noted the predominance of α-pinene, limonene and (Z)-β-ocimene [16] and shown correlation between chemical composition and the sampling sites.

In continuation of our contribution in knowledge this species essential oil compositions, this paper describes for the first time chemical investigation on essential oil from roots, collected to distinct localities from Côte d'Ivoire. Besides, Statistical analysis (PCA and CAH) was performed on chemical compositions obtained from different organs of *Tithonia diversifolia*, in order to establish the link between different organs.

2. Materiel and Methods

2.1. Materiel

The essential oils are extracted from different plant organs (leaves, flowers, stems, and roots) of *Tithonia diversifolia*. All the organs collected from different locations have been identified at the National Floristic Center of Félix Houphouët-Boigny University (Côte d'Ivoire). The sampling of *Tithonia diversifolia* have been done in four locations: Gagnoa (6°07' 54'' N 5°57' 02'' W), Sikensi (5°40' 34'' N 4°33' W), Attinguié (5°28' 00'' N 4°11' 00'' W) and Adjamé (5°21' 0'' N 4°1' 60'' W).

2.2. Methods

Ten milligrams of essential oil are dissolved in 100 mL of hexane and analyzed by GC-MS (Agilent Santa Clara CA USA). For each essential oil. This manipulation was repeated in three times. GC-MS was performed using an Agilent 7890B GC system (Agilent Santa Clara CA USA) equiped with a split-less injector and coupled with an Agilent SMD 5977B detector. One microliter of 0.01% essential oil solution was injected and the analytical conditions were set as follows:

1) Injection mode: splitless at 300 ℃ with a HP-5MS capillary column (Agilent Santa Clara CA USA) (30 m x 0.25 mm. df = 0.25 µm);
2) Temperature program: from 50 ℃ (1 min) to 300 ℃ (5 min) at a rate of 5 ℃/min.

Carrier gas: helium at a flow rate of 1.2 mL/min. Mass spectra were recorded in electron ionization mode at 70 eV (mass scan range: 40-400 m/z). Source and quadrupole temperatures were set at 230 ℃ and 150 ℃ respectively. Identification of components was based on chromatographic retention indices (RI) and comparison of recorded spectra with a computerized data library (Pal 600K®) [17, 18]. RI values were measured on an HP-5MS column (Agilent Santa Clara CA USA). RI calculations were performed in temperature program mode. A mixture of n-alkane homologues (C7-C30) was used under the same chromatographic conditions. Major components were confirmed by comparing their retention and MS spectrum data with co-injected pure references (Sigma Darmstadt Germany) when commercially available.

3. Results and Discussion

3.1. Yields

The results shown that EO yields varied between 0.043 to 0.059% for leaves, 0.01 to 0.035% for stems and 0.013% to 0.035% for roots. The flower EO yield varies between 0.033 to 0.035%. However, the leaf EO yield from Attinguié (0.059%) is higher than the other three locations. The yield (0.035%) from stems collected in Sikensi is higher than in other cities. On the other hand, the yield from Attinguié roots (0.013%) is lower than the three other locations. Leaf yields obtained in previous works by Brou et *al.* [16] (0.02-0.26%) in Côte d'Ivoire, by Lamaty et *al.* [19] (0.1%) in Cameroon and by Lawal et *al.* [20] in South Africa (0.21%) are higher than those obtained in our samples (0.043 to 0.059%). For stem yields, our values (0.01 to 0.035%) are higher than those obtained by Brou et *al.* [16] (0.01 to 0.017%). In the case of flowers, yields obtained by Chukwuka et *al.* [21] (0.01 to 0.063%) are higher than our yields (0.013 to 0.022%). Roots yield in South Africa (0.08) was higher than our samples (0.013% to 0.035%) [20].

3.2. Chemical Composition of Essential Oils of *Tithonia diversifolia*

3.2.1. Leaves Essential Oil Composition from *Tithonia diversifolia* Harvested in Attinguié, Adjamé, Gagnoa and Sikensi

Chemical composition of EO samples of the leaves from Attinguié (AT), Adjamé (AD), Gagnoa (GA) and Sikensi (SI) show 23 compounds corresponding respectively to 98.51 to 99.5% of the total chemical composition (Table 1).

Significant levels of hydrogenated monoterpenes are predominated in these samples, with the most important being *α*-pinene (8.66 to 29.76%), limonene (8.43 to 49.02%) and *trans*-*β*-ocimene (18.05 to 28.35%), whith dehydro-aromadendrene (2.02 to 8.16%), modephene (0 to 7.59%), spatulenol (0 to 7.08%), *cis* *α*-bisabolene (0 to 6.5%), 3-carene (0 to 6.06%), (*E*)-*β*-caryophyllene (0.41 to 6.06%) and caryophyllene oxide (2.2 to 5.08%). It should be noted that 3-methyl-2-(2-methybuthenyl) furan, germacrene D, eucalyptol, camphor, humulene and piperitone present in previous studies, are absent in our samples. *β*-pinene was the major compound in EOs from Brazil [14], Nigeria [21, 22], South Africa [20] and Kenya [13] with respective values of 21.9%; 10.9%; 10.7%; 15%, is present in low proportions in our samples (4.58%). Furthermore, in the previous study carried out in Côte d'Ivoire [16], *α*-pinene, limonene and *trans*-*β*-ocimene had relatively higher content (47.4%; 65.5%; 35.9%) in comparison whith the present study. This could be due to the edaphic factor impact.

3.2.2. Stems Essential Oil Composition from *Tithonia diversifolia* Harvested in Attinguié, Adjamé, Gagnoa and Sikensi

A total of 14 compounds have been identified from the analysis of all 8 EO samples of *Tithonia diversifolia* stems, representing 98.90-99.43% of the total chemical composition (Table 1).

Essential oils are dominated by hydrocarbon monoterpenes (83.45 to 99.10%). The predominant compound is *α*-pinene (68.4 to 88.03%), allowed by *β*-pinene (2.78 to 15.05%) and limonene (7.39 to 14.29%). Eucalyptol (0 to 9.83%) is the major component of oxygenated monoterpenes (13.02 to 14.09%).

Most important compounds such as trans-*β*-ocimene, spathulenol and limonene reported in our previous paper [16] with respective values of 39.3%, 11.8% and 36.5% are absent in those samples.

α-pinene present more abundant proportion in the EO from Gagnoa (88.03%) than those from Sikensi (80.05%), Adjamé (75.99%) and Attinguié (68.4%). However, *β*-pinene is dominant in Attinguié (15.05%) in comparison with Adjamé (6.29%), Sikensi (4.50%) and Gagnoa (2.78%). Moreover, *α*-pinene content (89.8%) obtained in previous studies [16], was close to that obtained in our essential oils (68.4 to 88.03%).

3.2.3. Flowers Essential Oil Composition from *Tithonia diversifolia* Harvested in Adjamé and Gagnoa

The EO of this species from Adjamé and Gagnoa revealed a total of 16 compounds (Table 1), representing 99.10 to 99.90% of each chemical analysis respectively.

Among the 16 compounds identified, monoterpene and sesquiterpene hydrocarbons can be observed. The proportions of hydrocarbons and oxygenated monoterpenes accounting from 79.79 to 97.83% and from 0.53 to 5.23% respectively. Among sesquiterpene hydrocarbons content ranges from 0.74 to 13.18% and oxygenated content from 0 to 1.7% of EO.

Indeed, samples from Gagnoa had most important monoterpene hydrocarbon (97.83%) than those from Adjamé (79.79%).

Monoterpene hydrocarbons were dominated by *α*-pinene, limonene and *trans*-*β*-ocimene. The main compound in flower samples was limonene. It was found a higher value in Gagnoa (33.08%) and Adjamé (40.07%). *α*-pinene proportion is higher in Gagnoa samples (42.04%) than Adjamé (35.08%). In Addition, *trans*-*β*-ocimene (18.93%) present in Gagnoa sample was absent in the Adjamé sample. For sesquiterpene hydrocarbons, *α*-cubebene (0.74 to 11.29%) was the dominant compound, with low proportion (0.74%) in Gagnoa samples than Adjamé (11.29%). Indeed, germacrene D (20.3%), *β*-caryophellene (20.1%), 1.8 cineole (8.76%) and bicyclogermacrene (8%) found in Nigerian samples were absent in our samples [22-23]. Limonene proportion in our samples was higher than those Cameroon [24].

3.2.4. Roots Essential Oil Composition from *Tithonia diversifolia* Harvested in Attinguié, Adjamé, Gagnoa and Sikensi

Chemical profile of EO obtained from *Tithonia diversifolia* shown 14 compounds corresponding to 99.5- 99.94% of the total chemical composition (Table 1).

It was dominated by hydrocarbon monoterpenes (61.82 to 98.86%) followed by sesquiterpenes hydrocarbon (1.02 to 34.8%) and oxygenated monoterpenes (0 to 3.18%). Oxygenated sesquiterpenes were absent in our samples. Monoterpene hydrocarbons were composed by *α*-pinene (51.04 to 97.01%) and *β*-pinene (1.46 to 10.05%). The main sesquiterpene hydrocarbon was modephene (1.08 to 15.77%). Content of *α*-pinene is significantly higher in the Gagnoa sample (97.01%) than this from Attinguié (51.04%), Adjamé (67.01%) and Sikensi (95.50%). Attinguié (10.05%) have higher proportion of *β*-pinene than Adjamé (1.46%), Gagnoa (1.47%) and Sikensi (1.69%). In the case of sesquiterpenes hydrocarbon, the modephene content is important in Attinguié (15.77%) and Gagnoa (14.59%). While it is low in Sikensi (1.08%). In South Africa samples, modephene was absent [20]. It was found that our EO was different from those cited in literature.

In addition, to our knowloledge, it was the first time that the roots essential oil was investigated in Côte d’Ivoire.

3.3. Chemical Variability of Essential Oil from Leaves, Roots and Stems of *Tithonia diversifolia*

The results of oil obtained from leaves, roots and stems from *Tithonia diversifolia* were submitted to hierarchical cluster, principal components analysis and discriminant factorial analysis. Its principal factors F1 and F2 accounted for 52.2%, of the total variance of chemical composition. Two groups were observable on the dendrogram from hierarchical cluster analysis (HCA) Figure 1. In addition, the principal component analysis (PCA) confirmed two groups from HCA Figure 2. Stems and roots samples were collected at Gagnoa, Sikensi, Attinguié and Adjamé (TTAT; TTAD; TTGA; TTSI; RTAT; RTAT; RTAD; RTGA and RTSI**)**, are constitued the group G1. This group is characterized by α-pinene, β-pinene, isocomene, modephene and eucalyptol Figure 3. However, leaves and flowers samples collected at Gagnoa, Sikensi, Attinguié and Adjamé (FTAT; FTAD; FTGA; FTSI; FlTAD and FlTGA), are composed the second group G2. It was dominated by *cis*-bisabolene, caryophyllene oxide, trans-ocimene, spatulenol, 3- carene, *(E)*β-caryophyllene, limonene, dehydroaromadendrene, cubene and spatulenol Figure 3.

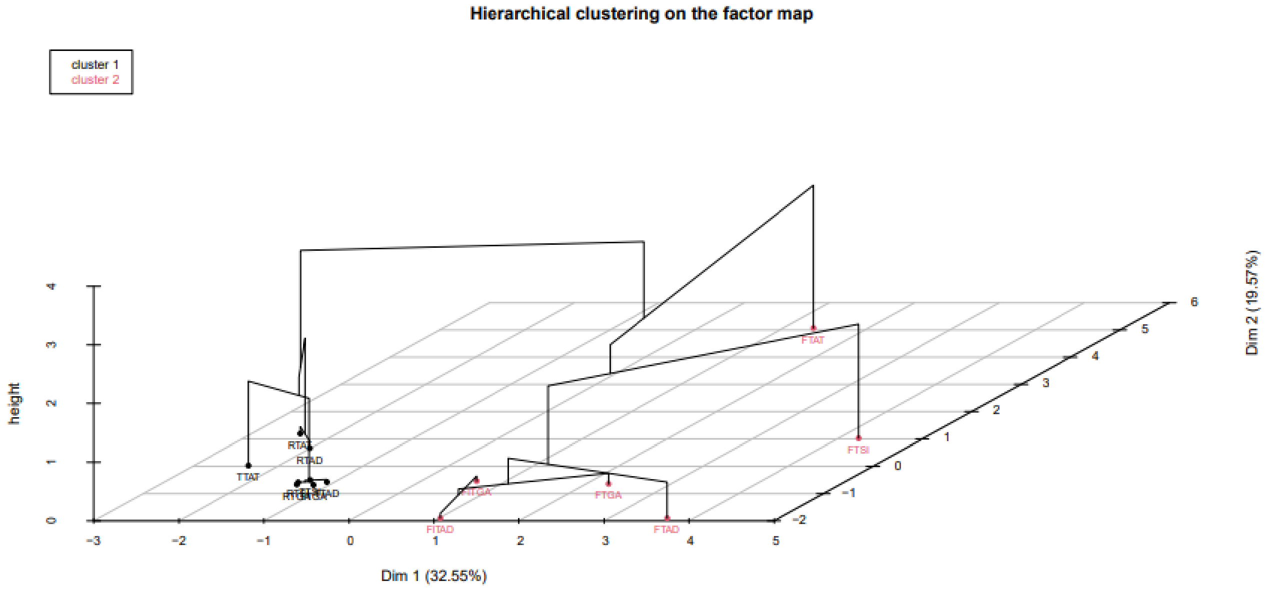

Figure 1. *Dendrogram obtained by hieraichical cluster analysis of leaf, flower, steams and roots oil from Tithonia diversifoli.*

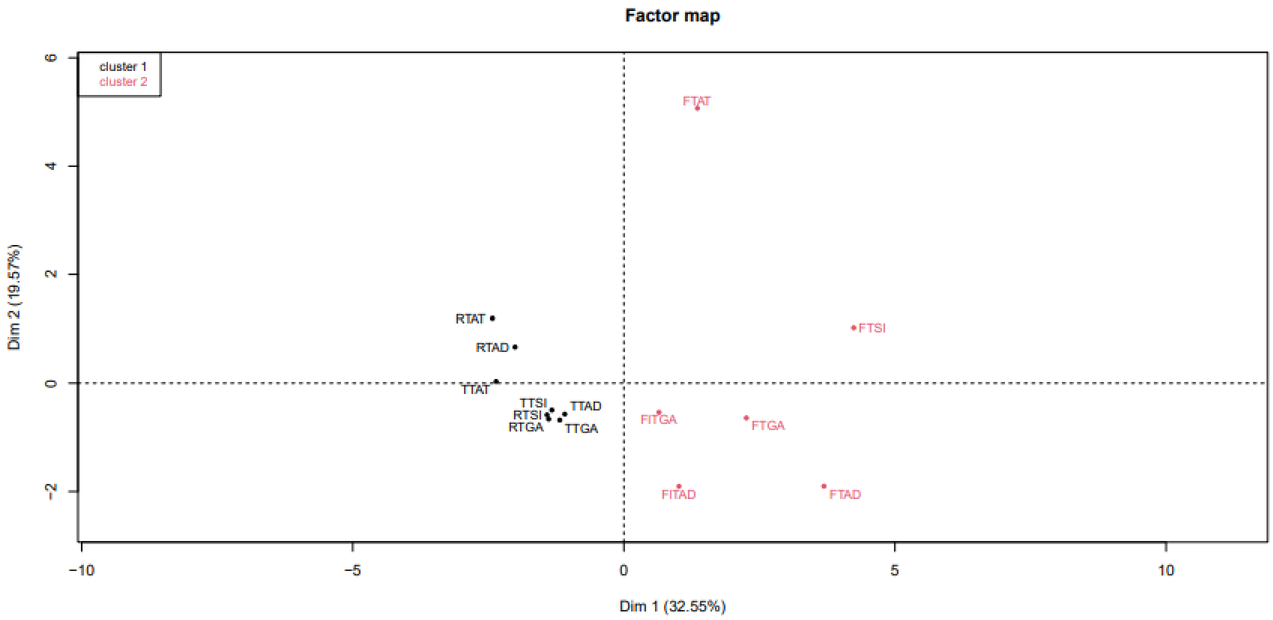

Figure 2. *Principal component analysis of leaf, flower, steams and roots oil from Tithonia diversifolia.*

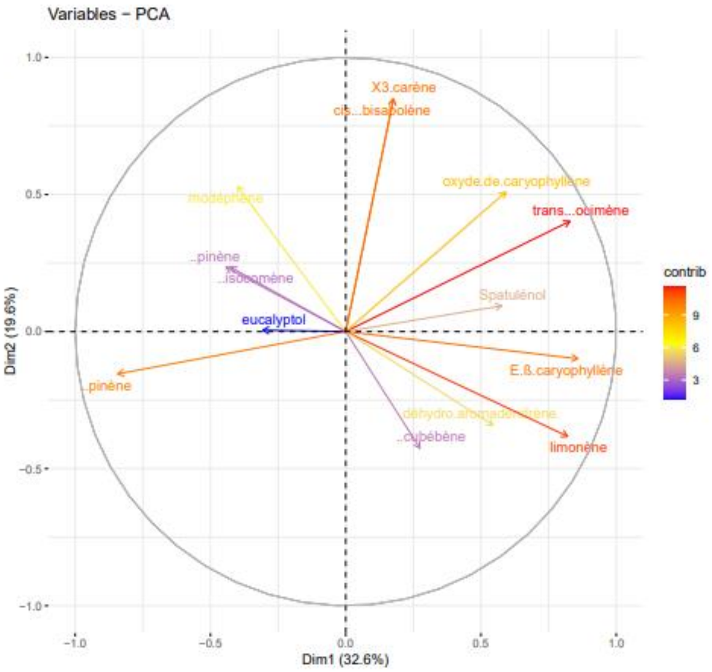

Figure 3. *Content components of leaves, steams, flowers and roots oil from Tithonia diversifolia.*

4. Conclusion

Leaves, stems, roots and flowers essential oils from *Tithonia diversifolia*, collected in Gagnoa, Attinguié, Sikensi and Adjame have been analysed by chromatographic and spectroscopic technique. The EO samples were rich in monoterpenes and sesquiterpenes hydrocarbons, as *α*-pinene, *β*-pinene, often following by limonen, *β*-phellandrene, (E) *β*- caryophyllene, modephene, trans-ocimene. In addition, statistical analysis exhibited distinct chemical group according to origin geographical and organs collected. It was found that the roots and stems of *Tithonia diversifolia*, were strongly linked and constitued the same group, when the second group is formed by the leaves and flowers. The chemical variability could be related to organ and edaphic factor.

Table 1. *Chemical composition of essential oil of leaves, stems, flowers, roots of Tithonia diversifolia from Attinguié, Adjamé, Gagnoa and Sikensi.*

				Leaves				stems				Flowers		Roots			
N°	**Compounds**	**Identi-fication**	**IK**	**FTAT**	**FTAD**	**FTGA**	**FTSI**	**TTAT**	**TTAD**	**TTGA**	**TTSI**	**FlTAD**	**FlTGA**	**RTAT**	**RTAD**	**RTGA**	**RTSI**
1	*α*-pinene	CPG. SM.IR	935	29.76	8.66	16.82	15.06	68.4	75.99	88.03	80.05	35.08	42.04	51	67.01	97	95.5
2	camphene	CPG. SM.IR	947					-	-	0.9	0.86	0.7	-	0.73	0.99	-	1.67
3	*γ*-terpinene	CPG. SM.IR	970	-	3.5	2.8	-					-	1.1				
4	*β*-phellandrene	CPG. SM.IR	973	-	-	-	2.04					2.22	-				
5	*β*-pinene	CPG. SM.IR	976	4.58	1.49	2.77	2.84	15.05	6.29	2.78	4.5	1.72	2.68	10.1	1.46	1.47	1.69
6	eucalyptol	CPG. SM.IR	1030					9.83	-	-	-						

				Leaves				**stems**				**Flowers**		**Roots**			
N°	**Compounds**	**Identi-fication**	**IK**	**FTAT**	**FTAD**	**FTGA**	**FTSI**	**TTAT**	**TTAD**	**TTGA**	**TTSI**	**FlTAD**	**FlTGA**	**RTAT**	**RTAD**	**RTGA**	**RTSI**
7	limonene	CPG. SM.IR	1031	8.43	49	44.06	26.18	-	14.29	7.39	-	40.07	33.08				
8	*trans-β*-ocimene	CPG. SM.IR	1037	28.35	18.1	27.02	25.58					-	18.93				
9	3-carene	CPG. SM.IR	1040	6.06	-	-	-										
10	cymene	CPG. SM.IR	1052	-	-	-	3.05	-	-	-	1			-	1.44	-	-
11	*trans*-verbenol	CPG. SM.IR	1107					-	-	-	3.74						
12	*α*-campholenal	CPG. SM.IR	1126											0.9	-	-	-
13	carveol	CPG. SM.IR	1144									-	0.53				
14	(1R)-cis-verbenol	CPG. SM.IR	1145					1.36	-	-	2.72	0.66	-	1.3	-	-	-
15	2-isopropylidene-3-methylhexa-3.5-dienal	CPG. SM.IR	1161									1.2	-				
16	*cis*-chrysanthenol	CPG. SM.IR CPG. SM.IR	1164									2.27	-				
17	pinocarvone	CPG. SM.IR	1164					0.7	-	-	1.1						
18	*cis*-myrtenal	CPG. SM.IR	1198					0.86	-	-	1.31						
19	L-verbenone	CPG. SM.IR	1211					1.34	-	-	3.06			0.98	-	-	-
20	1.2: 8.9-diepoxy-p-menthane	CPG. SM.IR	1262					-	-	-	1.09						
21	thymol	CPG. SM.IR	1327									1.1	-				
22	(-)-aristolene	CPG. SM.IR	1344											2.61	1.81	-	-
25	*α*-cubebene	CPG. SM.IR	1380	-	3.55	-	-	-	0.8	-	-	11.29	0.74	0.91			
26	modephene	CPG. SM.IR	1384	7.59	-	-	-	1.36	1.63	-	-			15.8	14.59	-	1.08
	α-isocomene	CPG. SM.IR	1391														
27	*β*-isocomene	CPG. SM.IR	1411											6.61	5.37	-	-

				Leaves				stems				Flowers		Roots			
N°	**Compounds**	**Identi-fication**	**IK**	**FTAT**	**FTAD**	**FTGA**	**FTSI**	**TTAT**	**TTAD**	**TTGA**	**TTSI**	**FlTAD**	**FlTGA**	**RTAT**	**RTAD**	**RTGA**	**RTSI**
28	*E*-ß-caryophyllene	CPG. SM.IR	1424	0.41	5.92	3.01	6.06										
29	*β*-cedrene	CPG. SM.IR	1426											0.47	-	1.02	-
30	*cis*-*α*-bisabolene	CPG. SM.IR	1458	6.5	-	-	-										
31	humulene	CPG. SM.IR	1458	0.35	-	-	0.3										
32	eremophilene	CPG. SM.IR	1493									0.89	-				
33	*β*-guaiene	CPG. SM.IR	1499	-	-	0.6	-										
34	bicyclogermacrene	CPG. SM.IR	1501	-	-	-	2										
35	*β*-curcumene	CPG. SM.IR	1504	-	-	-	-										
36	*δ*-cadinene	CPG. SM.IR	1525	0.7	1.15	-	-					1					
38	nerolidol	CPG. SM.IR	1573	1.1	-	-	1.11										
39	dehydro-aromadendrene.	CPG. SM.IR	1582	-	8.16	2.02	-										
40	Spatulenol	CPG. SM.IR	1584	-	-	-	7.08					1.7	-				
41	oxyde de caryophyllene	CPG. SM.IR	1590	2.2	-	-	5.08										
42	isospatulene	CPG. SM.IR	1645	2.67	-	-	0.53										
43	neointermedeol	CPG. SM.IR	1663	-	-	-	1.6										
Monoterpenes hydrocarbons (%)				77.18	80.7	93.47	74.75	83.45	96.57	99.1	86.41	79.79	97.83	61.8	70.9	98.5	98.9
Monoterpenes oxygenated (%)				0. 00	0	0	0	14.09	0	0	13.02	5.23	0.53	3.18	0	0	0
Sesquiterpenes hydrocarbons (%)				18.22	18.8	5.63	8.89	1.36	2.43	0	0	13.18	0.74	34.8	28.8	1.02	1.08
sesquiterpenes oxygenated (%)				3.3	0	0	14.87	0	0	0	0	1.7	0	0	0	0	0
Total (%)				98.7	99.5	99.1	98.51	98.9	99	99.1	99.43	99.9	99.1	99.8	99.7	99.5	99.9

Identification methods: IK. Theoretical Kovat indices (Pubchem and NIST); SM. Comparison of mass spectra with PAL 600® libraries; STD. comparison of retention time and mass spectra with commercially available standards; IR. comparison of retention index with literature; reference; -. Under perception threshold

Abbreviations

TTAT	Stems Sample from Attinguié
TTAD	Stems Sample from Adjamé
TTGA	Stems Sample from Gagnoa
TTSI	Stems Sample from Sikensi
RTAT	Roots Sample from Attinguié
RTAD	Roots Sample from Adjame
RTGA	Roots Sample from Gagnoa
RTSI	Roots Sample from Sikensi
FTAT	Leaves Sample from Attinguié
FTAD	Leaves Sample from Adjamé
FTGA	Leaves Sample from Gagnoa
FTSI	Leaves Sample from Sikensi
FlTAD	Flowers Sample from Adjamé
FlTGA	Flowers Sample from Gagnoa

Conflicts of Interest

The authors declare no conflicts of interest.

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