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Chemical variability of essential oils from the leaves of *Tridax procumbens* Linn (Asteraceae) from five cities of Côte d'Ivoire

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ABSTRACT

Chemical variability of a plant from various continents or collected in different localities is well known and justifies its different biological activities. Thus, the present study was undertaken to evaluate the chemical variability of essential oils from leaves of *Tridax procumbens* Linn from five localities of Cote d'Ivoire. Essential oils have been extracted by hydrodistillation with yields varying from 0.077 to 0.079% depending on the locality. Gas chromatography and mass spectrometry analysis of essential oils showed a predominance of thymol (20.9 to 68.9%) in the oils from all the localities except that from Yamoussoukro which is of an α-acoradiene (28.9%) predominance. Several of the most important and variously distributed minor compounds consist of p-cymene (2.2 to 11.3%), β-caryophyllene (1.5 to 6.0%), β-selinene (1.8 to 10.0%), α-selinene (0 to 7.3%), elemol (0 to 16.0%), α-eudesmol (0 to 7.5%), 6,10,14-Trimethylpentadecan-2-one (0.9 to 7.2%) and phytol (0 to 7.2%). The comparative study of the chemical compositions of essential oils showed that thymol is a marker of the plant. *Tridax procumbens* acclimated in Côte d'Ivoire have been grouped into three chemotypes of thymol and other compounds.

Keywords: Essential oils, *Tridax procumbens*, Chemical variability, Thymol, Chemical composition

INTRODUCTION

Plants are important sources of bioactive molecules and play an important role in the health of populations around the world, particularly in Africa (Soro et al., 2012; Goly et al., 2017; Ouattara et al., 2020). Among the many plants used in traditional medicine, the extracts of *Tridax procumbens* Linn (Asteraceae) have anticancer (Sankaranarayanan et al., 2013), healing (Babu et al., 2003), immunomodulatory (Oladunmoye, 2006), antioxidant (Pande et al., 2014), antimicrobial (Jindal and Kumar, 2012), antihypertensive (Ikewuchi et al., 2011), insecticide, antidiarrheal, antiviral, antibiotic (Sharma and Kumar, 2009), antidysentery, hypotensive, anti-inflammatory, hepatoprotective, immunomodulatory, wound healing (Sneha et Ruchi, 2010; Ahossi et al.,

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2014), antiulcer (Asula et al., 2015) and antidiabetic activities (Desai et al., 2015).

Phytochemical screening of non-volatile extracts of *Tridax procumbens* Linn has indicated the presence of alkaloids, carotenoids, flavonoids (catechins and flavones), tannins, fumaric acid, fluorinated sitosterol and saponins (Manjamalai et al., 2010; Vaishali and Rupali, 2015). The plant is rich in ions such as sodium, potassium and calcium (Mohammed et al., 2001). The leaves of *Tridax procumbens* Linn contain 35% crude protein, 6% crude fiber, 51% total carbohydrates and 6% crude lipid (Jude et al., 2009). *Tridax procumbens* L. also possesses phytotoxic compounds linked to its invasive nature and weed capacity (Mecina et al., 2016).

Essential oils, which are natural volatile substances found in a variety of plants, are used as pharmaceuticals, as flavor enhancers in many food products, as odorants in fragrances, and as insecticides. The essential oil of the leaves of *Tridax procumbens* Linn contains 15 compounds namely α-pinene, 1,3,6-octatriene, Camphene, β-pinene, Sabinene, Phellandrene, L-limonene, β-ocimene, Trans-β-ocimene, Trans-Caryophyllene, γ-elemene, Spathulenol, Torreyol and Aromadendrene (Manjamalai et al., 2012a). Essential oil from leaves of *Tridax procumbens* Linn induces Apoptosis and Suppresses Angiogenesis and Lung Metastasis of the B16F-10 Cell Line in C57BL/6 Mice (Manjamalai et al., 2012a), and possesses antimicrobial, anti-inflammatory (Manjamalai et al., 2012b), and antifungal activities (Manjamalai et al., 2012c).

Despite its interesting biological properties, the essential oil of *T. Procumbens* has been very low studied and the three articles found in the literature relate only to the Indian species.

Therefore, the present study aimed in evaluating the chemical variability of essential oils from leaves of *Tridax procumbens* Linn from five localities of Cote d'Ivoire.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Tridax procumbens* Linn were collected from July to September 2018 during the rainy season in five cities (Table 1) in center and west-center of Côte d'Ivoire. After identification by Mr. Amani N'GUESSAN, botanist of the National Polytechnic Institute Félix HOUPHOUËT-BOIGNY of Yamoussoukro (Côte d'Ivoire), the plant material collected was washed with running water and dried in the laboratory at room temperature (27 ± 2 °C), out of direct sunlight, for ten days (Haloui et al., 2015).

Extraction of essential oil

Essential oil was produced by hydrodistillation of dried leaves in a Clevenger apparatus according to the method described by Goly et al. (2015). Indeed, 300 g of dried leaves was introduced into a pressure cooker containing distilled water. The mixture was boiled using a heating mantle. The steam of water loaded with essential oil was condensed in the coil of the Clevenger, using a water flow. Three hours after the appearance of the first drop of the distillate, the essential oil was separated from water and dried on anhydrous magnesium sulfate (Merck, Germany). The collected oils were then stored at 4 °C, protected from light in a sealed opaque vial. Each extraction was procedured thrice and yield of essential oil was determined by the ratio of the mass of the extracted oil and the mass of the treated leaves using the formula: R (%) = m / M x 100; R = yield (%); M = mass of the dried leaves (g) and m = mass of the essential oil after 3 hours of distillation (g).

Gas Chromatography and Mass spectrometry

An Agilent 7890B GC system coupled to a MSD 5977B detector (Agilent, Santa Clara, CA, USA) and fitted with a HP-5MS capillary column (5% phenyl-95% methyl siloxane, 30 m x 0.25 mm, x 0.25 µm) was used, with helium as the carrier gas (1.2 mL/min). One µL of a solution of essential oil in hexane was injected in splitless mode. The temperature was programmed from 50 °C (1 min) to 300 °C (5 min), at a rate of 5 °C/min. The mass selective detector was operated with an ionization energy of 70 eV used over a scan mass range of 40–400 atomic mass units.

Table 1: GPS coordinates of the five study cities.

Towns	GPS coordinates	
	Latitude (N)	**Longitude (W)**
Daloa	6°52’36.085’’	6°27’5.794’’
Gagnoa	6°8’50.026’’	5°56’41.305’’
Guibéroua	6°14’19.947’’	6°10’16.692’’
Issia	6°39’13.425’’	5°36’20.001’
Yamoussokro	$6^0$47’18.762”	$5^0$15’25.9992”

GPS: Global Positioning System; N: North; W: West

The source and quadrupole temperatures were fixed at 230 °C and 150 °C, respectively. Data were analyzed using Mass Hunter Workstation Software, Qualitative Analysis Navigator and Qualitative Analysis Workflows (Version B.08.00, Agilent Technologies, Inc. 2016), with identification of the individual components based on their chromatographic retention index (RI) and comparison of spectra with a library (Pal 600K®). The RIs were experimentally determined using a series of C7–C30 n-alkanes and were compared with those reported in the literature (Babushok et al., 2011). Identifications were also made by reference to authentic standard compounds (Sigma, Darmstadt, Germany) analyzed under the same conditions as the essential oils, when they were commercially available.

RESULTS

Yields of essential oils

A preliminary kinetic study of the yield as a function of the drying time of leaves harvested at Yamoussoukro was carried out. The results of this study are given in Figure 1.

Results in Figure 1 show that the yields of essential oils are increasing until the tenth drying process day and then decrease from the eleventh day.

Chemical composition of essential oils

The chemical compositions of the essential oils of the leaves of *T. procumbens* from the five localities are given in Table 2.

From Table 2, we can notice that essential oils of *T. procumbens* have variable chemical compositions in function of cities. In general, thymol is present in all the essential oils from all localities in contrast with a total of 24 compounds identified in essential oils varying in content between 0.7 and 69.9% if present. Six major compounds, namely p-cymene, thymol, α-acoradiene, β-selinene, elemol and phytol, have contents greater than 9%. Essential oil of Daloa contains three major compounds: thymol (24.9%), elemol (16%) and β-selinene (10%). Those from Guibéroua, Gagnoa and Issia contain an only major compound which is thymol in proportions of 68.9, 64.1 and 64.6% respectively. Essential oil from Yamoussoukro contains four major compounds: α-acoradiene (28.94%), thymol (20.85%), p-cymene (11.29%) and phytol (9.36%). P-cymene, α-acoradiene and phytol, predominant in essential oil of Yamoussoukro, are poorly represented in essential oils from other localities. β-selinene and elemol, the major compounds in essential oil from Daloa, are poorly represented in essential oils from other localities.

The minor compounds of essential oils are ϒ-terpinene (1.90%), 4-terpineol (3.40%), β-citronellol (2.60%), citronellyl acetate (1.30%), β-elemene (0.90 - 4.40%), β-caryophyllene (1.50 - 6.01%), α-ionone (0.70 - 2.11%), α-humulene (1.10 - 2.90%), β-ionone (5.97%), α-selinene (1.50 - 7.29%), valencene (0.70 - 3.60%), δ-cadinene (0.80 - 4.50%), caryophyllene oxide (0.70 - 1.41%), veridiflorol (1.00%), ϒ-eudesmol (1.40 - 1.70%), ζ-cadinol (1.10 - 2.10%), β-eudesmol (1.20 - 1.80%), α-eudesmol (4.60 - 7.50%) and 6,10,14-Trimethyl pentadecan-2-one (0.90 - 7.20%).

Among these minor compounds, β-citronellol and citronellyl acetate / ϒ-terpinene, 4-terpineol, veridiflorol / β-ionone are specifically present in essential oils from Daloa / Guibéroua / Yamoussoukro respectively, while ϒ-eudesmol, ζ-cadinol, β-eudesmol and α-eudesmol are only present in essential oils from Daloa and Issia.

Compounds from essential oils can be classified into six families: Monoterpene hydrocarbons, Oxygenated monoterpenes, Sesquiterpene hydrocarbons, Oxygenated sesquiterpenes, Diterpenes and Others. The Figure 2 gives a breakdown of these families in each essential oil according to the localities.

Figure 2 shows that, in general, essential oils of *T. procumbens* from different localities of Côte d'Ivoire are mainly rich in Oxygenated monoterpenes. The essential oil from Daloa mainly contains Sesquiterpene hydrocarbons (35.5%), Oxygenated sesquiterpenes (29.2%) and Oxygenated monoterpenes (28.8%). Those from Guibéroua, Yamoussoukro and Gagnoa mainly contain Oxygenated monoterpenes (72.3%, 20.9% and 64.1% respectively) and Sesquiterpene hydrocarbons (14.9%, 46.2% and 22.1% respectively). The essential oil from Issia mainly contains Oxygenated monoterpenes (64.6%) and Oxygenated sesquiterpenes (15.1%). Monoterpene hydrocarbons (2.2 - 11.3%), Diterpenes (0 - 9.4%) and Others (0.9 - 10.9) are weakly present in all the essential oils.

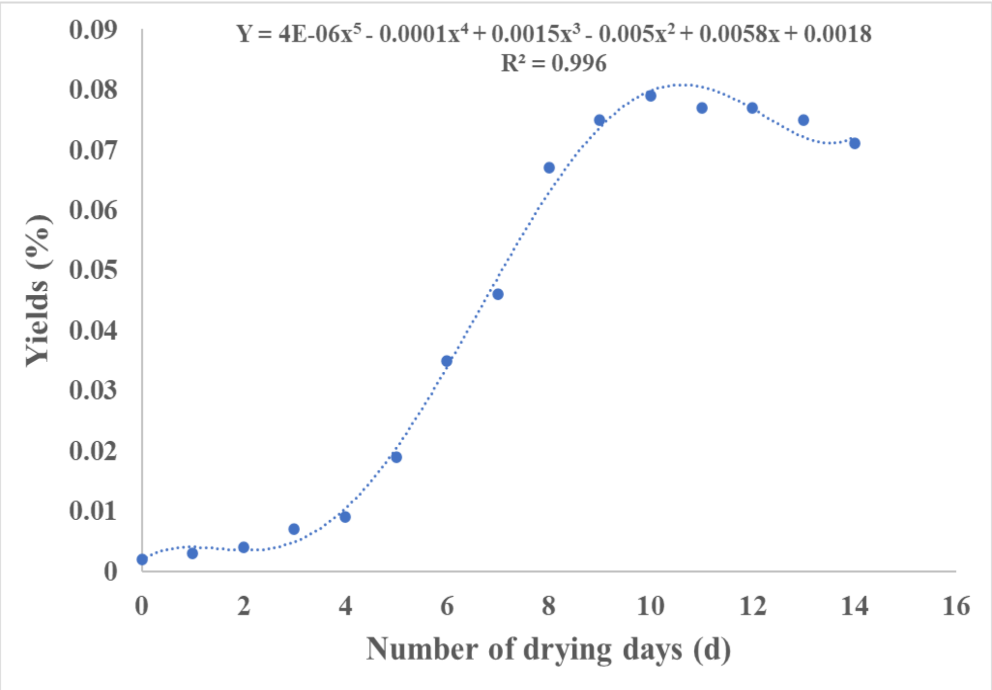

Figure 1: Yields of essential oil from fresh leaves of *Tridax procumbens* Linn as a function of drying days.

Table 2: Components of essential oils of *T. procumbens* from five localities of Côte d'Ivoire.

Compounds	KI theo	KI Cal	Towns				
			Daloa	**Guibéroua**	**Yamoussoukro**	**Gagnoa**	**Issia**
p-cymene	1026	1023	3.5	**6.8**	**11.29**	3.3	2.2
ϒ-terpinene	1060	1057	-	1.9	-	-	-
4-terpineol	1177	1177	-	3.4	-	-	-
β-citronellol	1217	1227	2.6	-	-	-	-
thymol	1297	1290	**24.9**	**68.9**	**20.85**	**64.1**	**64.6**
citronellyl acetate	1354	1353	1.3	-	-	-	-
β-elemene	1394	1393	4.4	-	-	-	0.9
β-caryophyllene	1417	1423	1.5	3.6	6.01	**6.0**	2.5
α-ionone	1429	1429	1.7	-	2.11	0.7	1.0
α-humulene	1456	1457	1.8	1.1	1.6	2.9	1.5
α-acoradiene	1464	1467	6.7	1.0	**28.94**	3.4	1.1
β-ionone	1486	1484.03	**-**	**-**	5.97	**-**	-
β-selinene	1488	1490	**10.0**	**5.7**	2.31	5.1	1.8
α-selinene	1494	1498	3.0	2.1	7.29	1.5	-
valencene	1495	1502	3.6	0.7	-	2.3	0.7
δ-cadinene	1519	1526	4.5	0.8	-	1.0	-
elemol	1550	1552	**16.0**	-	-	-	**6.9**
caryophyllene oxyde	1578	1589	-	0.7	1.41	-	-
veridiflorol	1587	1597	-	1.0	-	-	-
ϒ-eudesmol	1631	1635	1.7	-	-	-	1.4
ζ-cadinol	1639	1645	2.1	-	-	-	1.1
β-eudesmol	1642	1655	1.8	-	-	-	1.2
α-eudesmol	1652	1658	7.5	-	-	-	4.6
6,10,14-Trimethyl pentadecan-2-one	1842	1845	1.3	0.9	2.86	**7.2**	**5.7**
phytol	2122	2114	-	1.6	**9.36**	2.7	2.8
Total identified compounds (%)			**100**	**100**	**100**	**100**	**100**

KI on HP-5MS capillary column
KI theo: Theoretical Kovats retention index; KI Cal: Kovats retention index calculated from the experimental retention index.

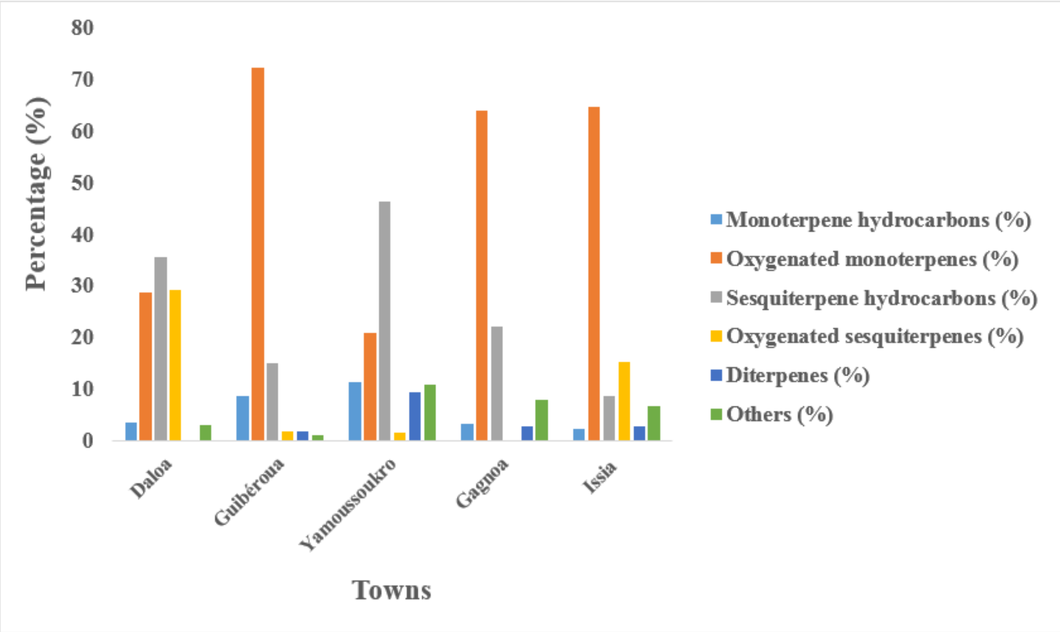

Figure 2: Distribution of the families of compounds from essential oils of *T. procumbens* from five localities of Côte d'Ivoire.

DISCUSSION

The results in Figure 1 showed that the highest yield (0.079%) was obtained on the tenth day of leaves drying. This drying time agrees with that of Haloui et al. (2015) but is greater than that of Tia et al. (2019). Therefore, after ten days of leaves drying, essential oils obtained were dark yellow. Their yields are, depending on localities, between 0.077 and 0.079%. We have not found in the literature extraction yields of essential oil of *T. procumbens* to compare them with ours.

In total, 24 compounds were identified in the essential oils of the leaves of *T. procumbens* from the five localities with detection proportions of 100% (Table 2). The city of Daloa was the richest in chemical compounds followed by Issia with 19 and 16 compounds respectively while the cities of Gagnoa and Yamoussoukro were the poorest in chemical compounds with only 12 compounds. As for the city of Guibéroua, it contains 15 compounds.

p-cymene, thymol, β-caryophyllene, α-humulene, α-acoradiene, β-selinene, α-selinene, 6,10,14-Trimethylpentadecan-2-one are present in essential oils from all the cities studied. Overall, thymol is the major compound of essential oils with contents varying from 20.85 to 68.9% except in essential oil from Yamoussoukro where α-acoradiene (28.94%) is the major compound. Four other compounds have contents greater than 9%. p-cymene is most abundant in essential oil from Yamoussoukro with content of 11.29%. β-selinene and elemol are more abundant in the essential oil from Daloa with content of 10.00 and 16.00% respectively. Phytol is more abundant in essential oil from Yamoussoukro with content of 9.36% (Table 2).

The richness of our oils in tymol brings them closer to essential oils of the genus Tymus also rich in tymol (Durnova et al., 2014; Nouasri et al., 2015) and of which several studies carried out on the genus Thymus have shown their antioxidant (Marina et al., 2014), antiseptic, carminative, antimicrobial, antifungal, antiviral, antiparasitic and spasmolytic activities (Stahl-Biskup and Venskutonis, 2004; Ghorab et al., 2013).

The results in Table 2 showed that essential oils from the five cities are different. They are also difference from essential oils of *T. procumbens* from India where only 15 compounds were identified with a detection proportion of 70%. The major compounds of this essential oil which were α-pinene (10.84%), 1,3,6-octa triene (20.90%), β-pinene (4.24%), Sabinene (6.98%), Phellandrene (9.64%) (Manjamalai et al., 2012b) are completely absent in our oils. However, α-Terpineol (1.7%), (*E*)-α-Ionone (2.6%), (*E*)-β-Ionone (1.2%), α-Selinene (15.3%) as well as α-Humulene, β-Acoradiene and α-Eudesmol present in trace form, have been identified in the flowers of *T. procumbens* from India (Joshi and Badakar, 2012). These results highlight the influence of the harvesting area on the composition of essential oils and could be linked to certain intrinsic factors such as climatic conditions and soil (Koffi et al., 2011).

The results in Figure 2 show that there is a predominance of Oxygenated monoterpenes in the essential oils of *T. procumbens* from Guibéroua, Gagnoa and Issia with content of 72.3, 64.1 and 64.6% respectively. Indeed, these oils are characterized by a high proportion of thymol (64.10 to 68.9%) and a negligible content of 4-terpineol (3.4%) for Guibéroua oil. The family of Oxygenated monoterpenes is also widely represented in oils of Yamoussoukro (20.9%) and Daloa (28.8%), the latter being characterized, in addition to thymol, by negligible contents of β-citronellol (2.6%) and citronellyl acetate (1.3%).

There is also a predominance of Sesquiterpene hydrocarbons in the essential oils from Daloa and Yamoussoukro with contents of 35.5 and 46.2% respectively. In fact, these essential oils are characterized by high proportions of α-acoradiene (6.70 and 28.94%), medium proportions of β-selinene (2.31 and 10.0%) and α-selinene (3.00 and 7.29%) as well as by low contents (1.5 to 6.0%) of β-elemene, β-caryophyllene, α-humulene, valencene and δ-cadinene. The family of Sesquiterpene hydrocarbons is present in significant quantities in the essential oils from Guibéroua (14.9%), Gagnoa (22.12%) and Issia (8.57%).

Oxygenated sesquiterpenes are widely represented in the essential oils of Daloa and Issia with contents of 29.2 and 15.1% respectively. They are characterized by significant proportions of elemol (16.0 and 6.9%) and α-eudesmol (7.5 and 4.6%) as well as by negligible percentages (1.1 to 2.1%) of ϒ-eudesmol, ζ-cadinol and β-eudesmol. This family is poorly represented in the essential oils of Guibéroua (1.66%) and Yamoussoukro (1.4%) with caryophyllene oxide and veridiflorol. However, no compound of the family of Oxygenated sesquiterpenes is present in essential oil from Gagnoa.

Overall, Monoterpene hydrocarbons are poorly represented in all essential oils from Daloa (3.5%), Guibéroua (8.6%), Gagnoa (3.3%) and Issia (2.2%) despite their average proportion of 11.3% in the essential oil of Yamoussoukro. The latter is characterized by p-cymene (2.2 to 11.29%) and ϒ-terpinene (1.9%).

Also, Diterpenes (1.64 to 9.40%) are poorly represented in the essential oils of Guibéroua (1.6%), Gagnoa (2.7%) and Issia (2.8%) and absent in essential oil of Daloa, despite their average proportion of 9.4% in essential oil of Yamoussoukro. This family is characterized by a single compound which is phytol (1.64 to 9.40%).

The other compounds are poorly represented in the essential oils of Daloa (3.0%), Guibéroua (0.9%), Gagnoa (7.9%) and Issia (6.7%) despite their average proportion of 10.9% in the essential oil of Yamoussoukro. These other compounds are characterized by α-ionone (0.7 to 2.1%), β-ionone (6.0%) and 6,10,14-Trimethylpentadecan-2-one (0.9 to 7.2%). These results are different from those of Manjamalai et al. (2012c) who found that the family of monoterpene hydrocarbons was the most abundant with content of 58.62% followed by the sesquiterpene hydrocarbons (5.85%) and the oxygenated sesquiterpenes (5.54%) in essential oil from leaves of *Tridax procumbens* from India.

A comparative study of the chemical compositions of essential oils of *Tridax* *procumbens* acclimated in Côte d'Ivoire show that thymol is a marker. The different *T. Procumbens* studied can be grouped according to thymol chemotypes and with other compounds. Thus, a first Chemotype consists of an essential oil rich in thymol, elemol and β-selinene corresponding only to the essential oil of Daloa. The second Chemotype consists of oils rich in thymol and moderately rich in p-cymene and β-selinene, or in 6,10,14-Trimethylpentadecan-2-one and in β-caryophyllene, or in elemol and in 6,10,14-Trimethylpentadecan-2-one and group the oils of Guibéroua, Gagnoa and Issia. The third Chemotype consists of an oil rich in thymol, in α-acoradiene and moderately rich in p-cymene, in phytol and corresponds to the essential oil of Yamoussoukro.

Conclusion

The first study of the chemical compositions of essential oils from the leaves of *Tridax procumbens* Linn from five cities in center and west-center of Côte d'Ivoire revealed new chemical compositions dominated by thymol and / or α-acoradiene. In general, the Oxygenated monoterpenes followed by the Sesquiterpene hydrocarbons were principal families of these essential oils. This study revealed that these essential oils can be classified in three chemotypes. However, chemotypes of essential oils from these cities are very different from that of India. These results highlight that essential oils of leaves of *T. procumbens* are strongly influenced by intrinsic factors such as climatic conditions and probably nature of soils. Studies are underway for evaluation of biological activities of obtained essential oils.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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