Comparative study of chemical variability of essential oils from the leaves of *Lippia multiflora* Mold (Verbenaceae) collected in five regions of Côte d'Ivoire

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ABSTRACT

Chemical variability of a plant from various continents or collected in different localities justifies its different biological activities. Thus, the present study was undertaken to evaluate the statistical study of chemical variability of essential oils from leaves of *Lippia multiflora* Mold from five localities in the center of Côte d’Ivoire. Essential oils have been extracted by hydrodistillation with yields varying from 0.34 to 1.20% depending on localities. Analysis of their chemical compositions showed the predominance of eucalyptol / germacrene D (13.91 / 11.02%), α-citral / β-citral / α-phellandrene (21.87 / 16.74 / 12.79%), linalool / (±)-trans-nerolidol / α-citral (19.99 / 18.68 / 12.10%), α-citral / β-citral (17.20 / 11.59%) and α-citral / β-citral (16.38 / 11.57%) in the oils from Yamoussoukro, Tiebissou, Bouaflé, Toumodi and Dimbokro, respectively. Principal Component Analysis and Hierarchical Ascending Classification revealed that these essential oils can be classified in three groups or chemotypes dominated by Eucalyptol, Citral / Linalool and trans-Geraniol.

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Keywords: Essential oils, *Lippia multiflora*, Terpene, chemical variability, statistical study.
been attributed to the presence of terpene compounds from its essential oil (Tia et al., 2011). Various studies on the chemical composition of essential oil in the sub-region revealed several chemotypes mainly dominated by Linalool and α-terpineol (Kanko et al., 2004) and nerolidol (Tia et al., 2011). *Lippia multiflora* appears as an essential oil plant with variable quality and quantity (Oussou et al., 2008) even within an ecological zone and for a given genotype with seasonal and environmental effects including those of the soil (Gazim et al., 2010; Diomandé et al., 2015).

Given the great variability in the composition of *Lippia multiflora* essential oil, it seems necessary to group the localities. In this context, the use of statistical methods such as Principal Component Analysis (PCA) and Hierarchical Ascending Classification (HAC) are important. PCA provides an opportunity to highlight the similarities and graphical position of two or more chemical variables as they evolve. Its use makes it possible to reduce and interpret data over a reduced space (Brereton, 2003). HAC is a more distinctive classification system where each member in a group is more similar to his colleagues than to any member outside the group (Güler et al., 2002). Therefore, the present study aimed of revealing a potential correlation between these localities.

**MATERIALS AND METHODS**

**Plant material**

The fresh leaves of *Lippia multiflora* Mold were collected at August 2019 during the rainy season in five cities in center of Côte d’Ivoire: Yamoussoukro (6°53′40″N 5°13′31″W), Tiébissou (7°08′18″N 5°13′36″W), Bouaflé (6°53′49″N 5°44′36″W), Toumodi (6°30′36″N 5°06′50″W) and Dimbokro (6°47′41″N 4°44′52″W). After identification by Mr. Amani NGUESSAN, botanist of the National Polytechnic Institute Félix HOUPHOÛET-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 four days (Tia et al., 2019).

**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, 500 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. Four 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: $R(\%) = 100 \times \frac{m}{M}$; $R = \text{yield (\%)}$ ; $M = \text{mass of the dried leaves (g)}$ and $m = \text{mass of the essential oil after 4 hours of distillation (g)}$.

The density, which represents the mass of the unit of volume at temperature T was determined according to standard ISO 6883: 1995 (NF 6883). The volumes of one (1) mL of dry oil and water were weighed. The masses of essential oil and the distilled water were used to determine the density (d) at room temperature of the laboratory (27 ± 2°C) according to the formula: $d = \frac{\rho_{huile}}{\rho_{eau}}$.

**Gas Chromatography and Mass spectrometry**

A Perkin Elmer Clarus 680 GC system coupled to an MSD 600C detector was used during the GC-MS analyses. The Rtx-SMS (Restek Co., Bellefonte, PA, USA) fused silica capillary column (30 m length x 0.25 mm i.d. x 0.25 μm film thickness) was used, with helium as the carrier gas (1 mL/min). One μL of an essential oil solution in hexane was injected in splitless mode with a ratio of 1:50. The temperature was programmed from 50°C (5 min) to 250°C (8.33 min), at a rate of
3°C/min for 80 minutes (total analysis time). The mass selective detector was operated with an ionization energy of 70eV and a scanning speed of 200 scans/min used over a scan mass range of 50–450 atomic mass units. The source and injector temperatures were fixed at 200°C and 250°C, respectively. The individual components were identified based on their retention indices on an Rtx-5MS capillary column under the same operating conditions used for GC–MSD, using a series of C7–C30 n-alkanes and were compared with those reported in the literature (Babushok et al., 2011). The structures were computer-matched with spectral libraries (Wiley 7, NIST 08, and FFNSC 1.2). Identifications were also made by reference to authentic standard compounds analyzed under the same conditions as the essential oils, when they were commercially available.

Statistical analysis
The results of chemical composition analyses of essential oils were processed by Principal Component Analysis (PCA) and Hierarchical Ascending Classification (HAC) (Landoulsi, 2016). The combination of these two methods makes it possible to know the differences or the similarities of the essential oils harvested in the different localities, in order to highlight a possible variability in their chemical compositions and to identify for each of the sites, the harvesting area corresponding to the maximum content of pharmacological interest compounds, then to highlight specificities within and between species. It was carried out using XLSTAT version 2014 software.

RESULTS
Yield and density of essential oils
Hydrodistillation of Lippia multiflora leaves from different localities yielded yellowish-coloured essential oils (Figure 1) characterized by an intense smell.

The average essential oil yields of the five (5) localities are shown in Figure 2. The yields vary from 0.34% for Yamoussoukro (LYK) to 1.2% for Toumodi (LTD). Essential oils densities vary from 0.73% for Bouaflé (LBF) to 0.94% for Toumodi (LTD). They are all less than 1.

Chemical composition of essential oils
The chemical compositions of the essential oils of Lippia multiflora leaves from the five localities are given in Table 1. KI on Rtx-5ms capillary column
KI theo: Theoretical Kovats retention index; KI Cal: Kovats retention index calculated from the experimental retention index.

GPC / MS analysis of Lippia multiflora essential oils from the five localities identified a total of eighty-three (83) constituents with overall proportions of 100% (Table 1). These compounds are divided into four (4) families: hydrocarbon monoterpenes, oxygenated monoterpenes, hydrocarbon sesquiterpenes and oxygenated sesquiterpenes.

Principal Component Analysis for the main compounds of Lippia multiflora essential oils from the five localities studied
The analysis of the links between chemical composition of essential oils and taxonomy of Lippia multiflora from the five localities was carried out by Principal Component Analysis (PCA). Only discriminating variables were considered. This analysis was based on 19 major chemical components of all essential oils with proportions greater than 1%. These are α-pinene, β-phellandrene, α-phellandrene, p-cimene, limonene + β-thujene, limonene, eucalyptol, linalool, α-terpineol, β-citral, trans-geraniol, α-citral, β-caryophyllene, α-caryophyllene, β-farnesene, germacrene D, γ-cadinene, (±)-transnerolidol and caryophyllene oxide. These compounds are fairly representative of all essential oil’s compounds with proportions of 89.81%, 93.75%, 94.61%, 96.48% and 96.22% for Yamoussoukro, Tiébissou, Bouaflé, Toumodi and Dimbokro, respectively.

The results of PCA used to study and visualize correlations between variables based on chemical similarities and differences are shown in Figure 3.

PCA score diagram represents 75.98% of the total variance in the dataset. The plane
formed by axes F1 and F2 gives correlation between the variables. The F1 axis (51.15% of the variation) is mainly constructed by positive correlation between $\beta$-farnesene, $\gamma$-cadinene, $\alpha$-terpineol, eucalyptol, $\beta$-phellandrene, caryophyllene oxide, germacrene D, $\beta$-caryophyllene and $\alpha$-caryophyllene with negative correlations between $\beta$-ocimene, trans-geraniol and limonene + $\beta$ thujene. The F2 axis (24.83% of the variation) is constructed by the positive correlation between $\alpha$-citral, $\beta$-citral, limonene, $\alpha$-phellandrene and $\alpha$-pinene with negative correlations between linalool and trans-nerolidol.

The resulting dendrogram provided a good picture of the relationships between the *Lippia multiflora* essential oil compounds from the five localities studied (Figure 4). The formation of the three chemical groups reveals an intraspecific variability between these oils.

**Hierarchical Ascending Classification**

Hierarchical Ascending Classification (HAC) also revealed the presence of three classes, the same, separated according to dissimilarity indices (Figure 5).

**Figure 1:** Photograph of *Lippia multiflora* essential oils.

**Figure 2:** Yield and density of *Lippia multiflora* essential oils from five (5) Ivorian localities.
Table 1: Components of *Lippia multiflora* essential oils from five localities of Côte d’Ivoire.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IK theo</th>
<th>IK Cal</th>
<th>LYK</th>
<th>LTS</th>
<th>LBF</th>
<th>LTD</th>
<th>LDk</th>
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2-(4a,8-Dimethyl-1,2,3,4a,5,6,8a-octahydro-2-naphthalenyl)-2-propanol

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

**Figure 3:** Principal Component Analysis of the main compounds of *Lippia multiflora* essential oils from the five localities studied.
Figure 4: Dendrogram of the analysis of *Lippia multiflora* essential oils composition.

Figure 5: Dendrogram of major compounds classification of *Lippia multiflora*.
DISCUSSION

The results in Figure 2 show that the best essential oil yield (1.2%) was obtained with the leaves harvested in Toumodi. This yield was followed by those of Tiébissou (0.94%) and Bouaflé (0.88%). The lowest essential oil yields were obtained with leaves from Dimbokro (0.44%) and Yamoussoukro (0.34%). The yield of essential oil from Yamoussoukro is lower than that obtained by Goly et al. (2017) (1.32%). However, yield of Toumodi (LTD) is higher than those of Soro et al. (2015) (0.76%) and Kanko et al. (2004) (0.8%). In general, variations in yield of essential oil can be attributed to several factors including species origin, harvest period, humidity level and plant development stage (Brada et al., 2007; Goly et al., 2017).

The essential oils densities of the different localities vary from 0.73 to 0.94 for the essential oils of Bouaflé and Toumodi, respectively. These densities are generally included in the essential oil density ranges (Bourkhiss, 2015).

A comparative study of the chemical compositions of essential oils from the five localities reveals similarities and differences. The results of Table 1 show that Yamoussoukro, Tiébissou, Bouaflé, Dimbokro and Toumodi contain 73, 54, 50, 34 and 29 compounds, respectively. Yamoussoukro is therefore the richest in compounds. Eucalyptol / linalool / α-citral / germacrene D in Yamoussoukro, α-phellandrene / β-citral / α-citral in Tiébissou and linalool / α-citral / β-citral / (±)-trans-nerolidol in Bouaflé are the main compounds of essential oils from the leaves of Lippia multiflora in these regions. β-citral, trans-geraniol and α-citral are the major compounds of the essential oil in the leaves of Toumodi and Dimbokro. These results therefore show a variability in the chemical composition of the essential oils from Lippia multiflora leaves in the five localities studied. Such variabilities have been reported for several plants including Leptospermum madidum (Demuner et al., 2011), Lippia multiflora (Soro et al., 2015) and Tridax procumbens (Coulibaly et al., 2020).

The essential oils of the five sites have oxygenated monoterpenes contents varying from 45.91 to 81.74%, the highest being obtained for Toumodi and Dimbokro. The second most abundant family in all the cities is that of hydrocarbon sesquiterpenes except for Tiébissou where hydrocarbon monoterpenes family occupies the second place. Oxygenated monoterpenes family is characterized by high proportions of eucalyptol (13.91%) / linalool (9.56%) / β-citral (6.89%) / α-citral (9.43%), β-citral (16.74%) / α-citral (21.87%), linalool (19.99%) / β-citral (9.44%) / α-citral (12.10%), β-citral (11.59%) / trans-geraniol (47.82%) / α-citral (17.20%), β-citral (11.57%) / trans-geraniol (49.43%) / α-citral (16.38%) in Yamoussoukro, Tiébissou, Bouaflé, Toumodi and Dimbokro, respectively. Significant rates of α-terpineol (3.63%) in Yamoussoukro, eucalyptol (4.02%) / linalool (2.22%) in Tiébissou, trans-geraniol (6.37%) in Bouaflé, linalool (4.05%) in Toumodi and linalool (2.89%) in Dimbokro are found in essential oils.

Hydrocarbon sesquiterpenes family is characterized by average proportions of β-caryophyllene (6.85%) / β-farnesene (6.09%) / germacrene D (11.02%), β-caryophyllene (6.06%) / β-farnesene (8.82%) / germacrene D (5.27%), β-caryophyllene (1.33%) / β-farnesene (4.53%) / germacrene D (2.43%) and β-farnesene (5.05%) / germacrene D (2.93%) in Yamoussoukro, Bouaflé, Toumodi and Dimbokro, respectively.

Hydrocarbon monoterpenes and oxygenated sesquiterpenes are only abundant in essential oils from Tiébissou and Bouaflé with proportions of 29.53 and 20.38%, respectively. Hydrocarbon monoterpenes of Tiébissou are dominated by α-phellandrene (12.79%), p-cimene (6.60%) and limonene (6.06%) while oxygenated sesquiterpenes of Bouaflé are dominated by (±)-trans-nerolidol (18.68%).

Essential oils obtained at the five sites have high monoterpenic content. This chemical characteristic is in accordance with the results obtained by Soro et al. (2015). In addition, these oils are highly concentrated in oxygenated monoterpenes, in agreement with
the results of Oussou et al. (2008). Eucalyptol, citral / linalool and trans-geraniol chemotypes obtained in this study complement or confirm the twelve (12) described by Adou et al. (2011). In general, variations observed in the chemical composition of essential oils can be attributed to several factors including soil characteristics (Diomandé et al., 2015).

Principal Component Analysis (PCA) (Figure 3) shows that the main *Lippia multiflora* essential oil compounds from the five locations studied are divided into three (3) groups. Group I, formed by Bouaflé (LBF) and Yamoussoukro (LYK), is characterized by the presence of β-farnesene, γ-cadinene, α-terpineol, eucalyptol, β-phellandrene, caryophyllene oxide, germacrene D, β-caryophyllene, α-caryophyllene, (±)-trans-nerolidol and linalool. Group II, formed by Tiébissou (LTS), is characterized by the presence of α-citral, β-citral, limonene, α-phellandrene and α-pinene. Group III, formed by Toumodi (LTD) and Dimbokro (LDK), is characterized by the presence of trans-geraniol and limonene + β-thujene.

Hierarchical Ascending Classification (HAC) also revealed the presence of three separate classes according to the dissimilarity indices (Figure 5). This aggregation method displays the different relationships between oil samples, as a dendrogram calculated using the Ward Minimum Variance method. HAC identified three chemical classes (Classes C1, C2 and C3) in all samples. Class C1 consists of 5 compounds from Yamoussoukro (LYK) (β-phellandrene, γ-cadinene, α-caryophyllene, caryophyllene oxide and α-terpineol), 3 compounds from Tiébissou (LTS) (α-pinene, β-ocimene and limonene), and 1 compound from Dimbokro (LDK) and Toumodi (LTD) (limonene + β-thujene). Class C2 consists of 3 compounds from Tiébissou (LTS) (β-farnesene, α-citral and β-citral) and 6 compounds from Yamoussoukro (LYK) and Bouaflé (LBF) (eucalyptol, linalool, α-phellandrene, germacrene D, β-caryophyllene and trans-nerolidol). Class C3 consists of 1 compound from Toumodi (LTD) and Dimbokro (LDK) (trans-geraniol).

Overall, oxygenated monoterpenes dominate the chemical composition of Group II (26.65%), while in Groups I and III, their percentages are 12.31% and 20.86%, respectively. The six major compounds (≥5%) are linalool, germacrene D, β-farnesene, trans-geraniol, β-citral and α-citral. These results clearly show that essential oils from the leaves belong to different chemical groups. We did not find similar studies on *Lippia multiflora* essential oils in the literature to compare them to our study. However, similar study on essential oils from *Pistacia atlantica* revealed two different chemotypes (one with α-pinene and the other with α-pinene / sabine / terpinen-4-ol) (Sifi et al., 2015) and three chemotypes including one in δ-3-carene (16.4-56.2%), another in terpinen-4-ol (10.8-16.0%) and a last in α-pinene / camphene (10.9-66.6 / 3.8-20.9%) (Gourine et al., 2010).

**Conclusion**

Chemical compositions study of essential oils from the leaves of *Lippia multiflora* Mold from five cities in center of Côte d’Ivoire revealed a variability in the chemical composition of the essential oils. In general, the oxygenated monoterpenes followed by the hydrocarbon sesquiterpenes were principal families of these essential oils. Principal Component Analysis and Hierarchical Ascending Classification revealed that these essential oils can be classified in three groups or chemotypes dominated by eucalyptol, citral /linalool and trans-geraniol. These results highlight those essential oils of leaves of *Lippia multiflora* are strongly influenced by intrinsic factors such as climatic conditions and probably nature of soils. The comparative chemical compositions study of essential oils showed that α-citral is a marker of *Lippia multiflora* acclimated in the center of Côte d’Ivoire.

**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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