Comparison of the structures and antioxidant activity of the major compounds of *Lippia multiflora* Mold (Verbenaceae) leaves essential oils collected in center of Côte d'Ivoire

Abstract: The growing interest in natural antioxidants is motivated by the harmful effects of the use of synthetic antioxidants. Medicinal plants such as *Lippia multiflora* are being studied for their multiple biological potentials, in particular their ability to provide new sources of natural antioxidants. The aim of this study was to determine the chemical composition and assess the antioxidant activity of *Lippia multiflora* essential oils from five (5) localities in Côte d'Ivoire. The essential oils were extracted by steam stripping and analyzed by gas chromatography-mass spectrometry (GC-MS) to determine their chemical composition. Their antioxidant activity was assessed using DPPH and ABTS tests.The analyses revealed that the major compounds in the essential oils from Yamoussoukro, Tiébissou, Bouaflé, Toumodi and Dimbokro were α-phellandrene, Eucalyptol (1,8-cineole), Linalool, β-citral (Neral), trans-geraniol, α-citral (Geranial), Germacrene D and (±)-trans-nerolidol, respectively. The best antioxidant activities for the DPPH and ABTS tests were obtained for Dimbokro and Toumodi essential oils respectively. These results suggest that *Lippia multiflora* essential oils have significant biological potential and could be used as an alternative to synthetic antioxidants, with possible applications in the agri-food and pharmaceutical industries.

Key words: Essential oils, *Lippia multiflora*, antioxidant activity, major chemical compounds.

1. INTRODUCTION

The genus *Lippia*, which belongs to the Verbenaceae family, comprises around 41 genera, 220 species of which grow in the tropical regions of West and Central Africa. Among them, *Lippiamultiflora*Moldenke is a shrubby aromatic plant that is widespread in West and Central Africa **(Pascual *et al.*, 2001; Jigamet*al.*, 2009; Owolabi *et al.*, 2009)**. In Côte d'Ivoire, *Lippia multiflora* is the only species represented, probably with numerous chemotypes **(Oussou *et al.*, 2008)**. This plant grows in the savannah, from the forest-savannah transition zone, known as the "V Baoulé" (Tiassalé Department), to the Lake and Central regions (Yamoussoukro, Dimbokro, Bouaké, Sakassou, Katiola), and to the North, North-West and North-East regions (Korhogo, Odienné, Séguéla, Bouna, Bondoukou) **(Aké Assi, 2009)**.

Lippia multiflora is used in traditional medicine to treat a variety of ailments, including malaria, diabetes, pain, flu, fever, gastrointestinal disorders, coughs and high blood pressure, and is also used

as a laxative. Infusions of it's leaves help to detoxify the body and burn fat **(Pascual *et al.*, 2001; Abena *et al.*, 2003; Okpekon*et al.*, 2004).**

The essential oil extracted from this plant has shown several biological activities, including antioxidant activity **(Kobenan *et al.*, 2022)**. Research has shown that the chemical composition of this oil varies according to locality, with a high terpene content **(Bassoléet *al.*, 2003; Simon *et al.*, 2008; Atché*et al.*, 2022; Owolabi *et al.*, 2009; Tia *et al.*, 2011; Soro *et al.*, 2015)**.

In Côte d'Ivoire, studies have been carried out on the impact of agro-ecological conditions on the chemical composition and biological properties of *Lippia multiflora* leaves grown in different ecological zones in the center of the country **(Abena *et al.*, 2003; Soro *et al.*, 2015)**. However, there are few studies on the variability of the chemical composition and antioxidant activity of essential oils of *Lippia multiflora* leaves harvested in Côte d'Ivoire. Thus, the aim of this study is to compare the major compounds and antioxidant activities of essential oils of *Lippia multiflora* leaves harvested in five (5) localities in central Côte d'Ivoire in order to establish a relationship between the structures of these compounds and their antioxidant activity.

2. MATERIAL AND METHODS

2.1. Plant material

Fresh leaves of *Lippia Multiflora* Mold were harvested in August 2019 during the rainy season in five (5) towns in central 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 N'GUESSAN, botanist at the Institut National Polytechnique Félix HOUPHOUËT-BOIGNY in Yamoussoukro (Côte d'Ivoire), the harvested plant material was washed in running water and dried in the laboratory at room temperature (27 ± 2°C), out of direct sunlight, for four days **(Tia *et al.*, 2019;Atché*et al.*,2022)**.

Figure 1: Fresh (A) and dried leaves of *Lippia multiflora* (B) **(Atché Photos: 15/08/2019)**

2.2 Essential oil extraction

The essential oil was produced by hydrodistillation of dried leaves in a Clevenger apparatus using the method described by **Goly *et al.* (2015)**. In fact, 500 g of dried leaves were introduced into a pressure cooker containing distilled water. The mixture was boiled using a heating jacket. The essential oil-laden water vapor was condensed in the Clevenger coil using a water stream. Four hours after the first drop of distillate appeared, the essential oil was separated from the water and dried on anhydrous magnesium sulphate (Merck, Germany). The collected oils were then stored at 4°C, protected from light, in a sealed opaque bottle.

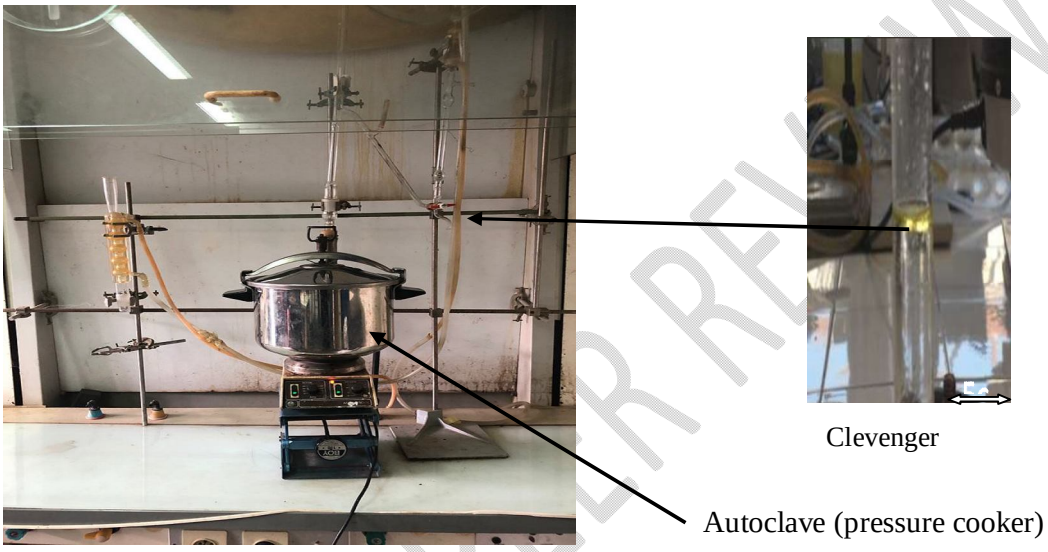

Figure 2: Device for extracting essential oils **(Atché photos: 24/08/2019)**

2.3 Gas chromatography and mass spectrometry

A Perkin Elmer Clarus 680 GC system coupled to an MSD 600C detector was used for GC-MS analysis. The Rtx-5MS fused silica capillary column (Restek Co., Bellefonte, PA, USA) (30 m length x 0.25 mm internal diameter 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 at 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 operated with ionization energy of 70 eV and a scan rate of 200 scans/min over a scan mass range of 50 to 450 atomic mass units. The source and injector temperatures were set at 200°C and 250°C, respectively.Individual components were identified on the basis of 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 **(Tia *et al.*, 2011; Soro *et al.*, 2015; Atché*et al.*, 2022)**. The structures were matched by computer 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.

2.4 Antioxidant activity

Two methods were chosen for their ease of use and reliability in assessing the antioxidant activity of essential oils. These were the 2,2-Diphenyl-1- picrylhydrazyl (DPPH) test and the 2,2-azino-bis-(3-ethylbenzothialozine-6-sulphonic acid) ABTS test.

2.4.1 Scavenging test for the free radical 2,2-Diphenyl-1- picrylhydrazyl

The capacity of essential oils from plant leaves to trap the DPPH free radical was assessed using the method described by **(Bicas*et al.*, 2011; Soumahoro *et al.*, 2023)**, followed by calculation of the IC50. The essential oil stock solutions were prepared in methanol at a concentration of 1000 µg/mL. A series of dilutions in geometric progression at a rate of 2 was carried out to obtain a range of concentrations (1000; 500; 250; 125; 61.5; 31.25 and 15.62 µg/mL) of the essential oils or vitamin C (reference or control antioxidant). 1 mL of each solution was mixed with 1 mL of a methanolic solution of DPPH (0.04% in methanol). The reaction mixture was shaken vigorously and incubated for 30 min in the dark at room temperature (20°C). Absorbance was measured at 517 nm. The experiment was performed in triplicate.

Antioxidant activity is expressed as the percentage of inhibition calculated using the formula in relation (8):

$$\mathbf{AntioxidantActivity(\%) = 100 \times [(A_{control} - A_{sample}) / A_{control}]} (8)$$

Where, $A_{control}$ and A_{Sample} represent the respective absorbance of the control sample and the essential oil.

The inhibitory concentration (IC_{50}) was calculated from the graph of antioxidant activity (in %) as a function of different concentrations of the essential oils tested.

2.4.2 Trapping test for the free radical 2,2-azino-bis-(3-ethylbenzothialozine-6-sulphonic acid)

The ABTS test was carried out according to the method described by **Re et al (1999)**. The ABTS solution (7 mM) is added topotassium persulphate solution (2.4 mM). The cationic radical **$ABTS^{\cdot+}$** is generated by mixing the two solutions in equivalent quantities. The reaction mixture was protected from light for 12 hours at room temperature (20°C). 1 mL of the resulting solution was diluted with 60 mL ethanol to give an absorbance of 0.8, measured using a spectrophotometer at 734nm. The percentage degradation of ABTS (A) was expressed by the formula in relation (9):

$$\mathbf{\% I = [(A_0 - Abs_{extract}) / A_0] \times 100} (9)$$

A_0 and $Abs_{extract}$ represent the respective absorbance of the diluted ABTS solution and that of diluted ABTS + sample.

3. RESULTS AND DISCUSSIONS

3.1 Chemical composition of essential oils

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)**.

Table 1: Chemical composition of the essential oil of *Lippia multiflora* leaves

Name of chemical compounds	KI theo	KI Cal	LYK	LTS	LBF	LTD	LDK
α-Thujene	925	927	0.14	0.23	0.05	-	0.06
α-Pinene	932	937	1.21	1	0.19	0.31	0.21
Camphene	945	946	0.04	-	-	-	-
1-Octen-3-ol	964	968	0.03	-	-	-	-
β-Pinene	972	978	0.45	0.09	-	-	-
3-Octanol	981	984	0.03	0.04	0.04	-	0.04
β-Myrcene	983	985	0.82	0.85	0.17	0.17	0.15
6-Methyl-5-heptene-2-one	985	988	0.18	0.17	0.26	0.2	0.26
***α*-Phellandrene**	998	1002	**3.61**	**12.79**	**3.21**	**1.66**	**2.83**
p-Cimene	1013	1014	2.45	6.6	1.15	1.42	1.14
Limonene+*β*-Thujene	-	-	-	-	-	1.14	-
β-Phellandrene	1021	1023	3.48	0.98	0.05	-	0.05
Eucalyptol	1022	1024	**13.91**	4.02	0.07	-	-
Limonene	1031	1031	1.34	6.06	1.3	-	1.28
β-Ocimene	1036	1037	0.63	0.87	0.29	0.91	1.35
Trans-*β*-Ocimene	1050	1048	-	-	0.03	-	-
γ-Terpinene	1051	1053	0.05	-	-	-	-
Terpinolene	1081	1082	0.1	0.07	-	-	-
Linalool	1085	1086	**9.56**	**2.22**	**19.99**	**4.05**	**2.89**
Isoterpinolene	1086	1083	-	-	0.01	-	-
Linalool oxide	1088	1088	0.01	-	-	-	-
Linalool, methylether	1098	-	0.21	-	-	-	-
α-Campholenal	1125	1128	0.07	0.03	0.01	-	-
(-/+)-*cis*Verbenol	1142	1142	0.51	0.67	0.35	0.31	0.27
Citronellal	1143	1145	0.03	0.03	0.03	-	-
(+ / -)-*trans*Verbenol	1150	1150	0.35	0.36	0.19	0.15	0.47
Cis-*β*-Terpineol	-	1159	0.22	0.04	-	-	-
Sabina cétone	1156	1159	0.06	-	-	-	-
Pinocarveol	1162	1164	0.1	-	-	-	-
4-Terpineol	1164	1166	0.12	0.03	-	-	-
α-Terpineol	1174	1175	3.63	1.18	0.05	-	-
Borneol	1176	1177	0.19	0.09	-	-	-
(-)-*cis*-sabinol	1179	-	0.13	0.3	0.05	-	-
Cryptone	1190	1188	-	0.12	0.02	-	-
***β*-citral ou Neral**	1215	1218	**6.89**	**16.74**	**9.44**	**11.59**	**11.57**
Trans-piperitol	1208	1220	0.04	0.06	0.02	-	-
Cis-carveol	1229	1222	0.04	-	-	-	-
Acetate de Nerol	1220	1223	-	-	-	0.59	-
Citronellol	1217	1227	0.05	0.03	0.05	0.11	0.04
Cis-géraniol	1236	1237	0.03	0.08	0.08	0.15	0.23
***α*-citral ou Geranial**	1243	1249	**9.43**	**21.87**	**12.1**	**17.2**	**16.38**
***Trans*-Geraniol**	1255	1259	0.08	0.6	6.37	**47.82**	**49.43**

Name of chemical compounds	KI theo	KI Cal	LYK	LTS	LBF	LTD	LDK
Thymol	1267	1270	0.05	0.1	-	-	-
Acétate de Bornyle	1279	1286	-	-	-	0.23	0.12
α-Cubebene	1353	1354	0.22	0.07	0.1	0.04	0.04
α-Bourbonene	1388	1378	0.22	0.06	0.12	-	-
β-Cubebene	1381	1385	0.28	0.1	0.14	0.08	0.06
β-Elemene	1392	1392	0.13	0.03	0.09	-	-
(+)-Sativene	1393	1396	0.03	-	-	-	-
Isoeugenol	1403	1404	0.01	-	-	-	-
β-caryophyllene	1425	1424	6.85	3.27	6.06	1.33	1.03
Copaene	1432	1432	0.7	0.29	0.33	0.2	0.17
α-bergamotene	1436	1438	0.03	-	0.03	-	-
Trans-chrysanthemal	1451	-	0.06	0.2	0.07	-	0.03
***β*-Farnesene**	1452	-	**6.09**	**8.86**	**8.82**	**4.53**	**5.05**
α-caryophyllene	1458	1458	1.83	0.5	1.26	0.2	0.19
γ-muurolene	1477	1474	0.18	0.04	0.06	-	0.02
Germacrene D	1483	1482	**11.02**	**4.06**	**5.27**	**2.43**	**2.93**
α-muurolene	1499	1498	0.32	0.11	0.16	0.07	0.04
8-Isopropenyl-1,5-dimethyl-1,5-cyclodécadiene	1503		0.13	-	0.09	-	-
β-Bisabolene	1509	1507	0.34	0.41	0.84	0.15	0.19
β-cadinene			-	-	0.55	-	-
γ-cadinene	1512	1513	1.09	0.46	-	0.26	0.21
β –Sesquiphellandrene	1524	1523	0.05	-	0.05	-	-
Hedycaryol	1530	-	0.11	-	0.08	-	-
(±)-*trans*-Nerolidol	1549	1552	**6**	**1.8**	**18.68**	**2.31**	**0.9**
Germacrene D-4-ol	1574	1579	-	0.06	0.16	-	0.03
Caryophyllene oxide	1578	1580	1.34	0.74	0.6	0.23	0.13
Guaiol	1595	1600	0.16	0.02	0.16	-	-
4-Isopropyl-1-methyl-2-cyclo hexen-1-ol	1605	-	0.08	0.17	0.06	-	-
β-Eudesmol	1630	1630	0.11	-	-	-	-
τ.-cadinol	1632	1634	0.08	0.02	-	-	-
τ-muurolol	1642	-	0.15	0.03	-	-	-
Cubénol	1646	1644	0.11	0.02	-	-	-
(-)-*δ*-cadinol	1643	1650	0.93	0.29	0.59	0.16	0.18
α-cadinol	1653	1656	0.13	0.02	-	-	-
Bulnesol	1666	-	0.07	-	-	-	-
Trans-Bergamotol,	1684	1700	0.37	-	-	-	-
Farnesol	1717	1726	0.02	-	-	-	-
E,E-Farnesal	1722	-	0.02	-	-	-	-
Hexahydrofarnesylacetone	1914	1916	0.07	-	-	-	-
2-(4a,8-Diméthyl-1,2,3,4,4a,5,6,8a-octahydro-2-naphthalen yl)-2-propanol	2224		0.36	0.05	0.1	-	-
Total compounds identified (%)			100	100	100	100	100
Hydrocarbonmonoterpenes			14.30	**29.53**	6.45	3.76	7.09
Oxygenated monoterpènes			**45.91**	**49.15**	**49.22**	**81.61**	**81.74**
Hydrocarbonsesquiterpenes			**29.55**	18.26	**23.96**	**9.47**	**9.53**
Oxygenatedsesquiterpenes			10.25	3.05	**20.38**	3.11	1.64

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

LYK: LOCALITY YAKRO; LTD: LOCALITY TOUMODI; LBF: LOCALITY BOUAFLE; LDK: LOCALITY DIMBOKRO; LTS: LOCALITY TIEBISSOU

There are eight (8) major compounds (whose percentage is greater than or equal to 10%). These are:α-phellandrene, Eucalyptol (1,8-cineol), Linalool, *β*-citral (Neral), trans-geraniol, *α*-citral (Geranial), Germacrene D and (±) -trans-nerolidol **(Figure 3)**.

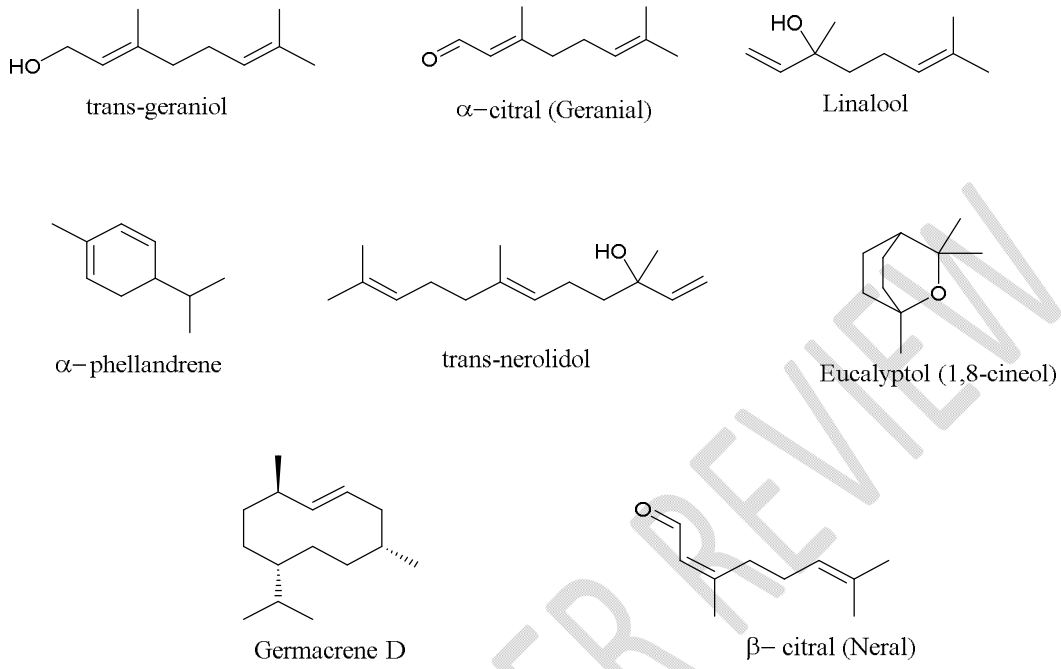

Figure 3: Structures of the main chemical compounds in the essential oil molecules of *Lippia multiflora* leaves from the five locations studied.

These major chemical compounds represent between 60.50 and 87.05% of all the compounds detected in the essential oils of the different localities. They are therefore representative of theessential oils from the different localities**(Figure 4)**.

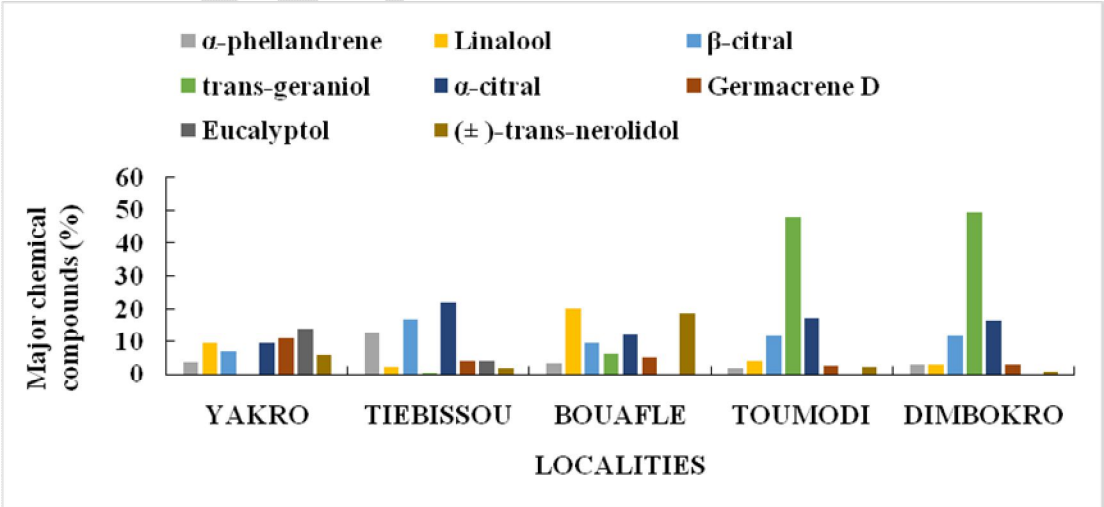

Figure 4: Distribution of the major chemical compounds in the essential oil of *Lippia multiflora* leaves according to locality

The essential oils of *Lippia multiflora* leaves from the five (5) localities also contain four (4) intermediate compounds (whose percentage is between 5 and 10%). These are: *β*-caryophyllene, limonene, *p*-cimene and *β*-farnesene**(Figure 5)**.

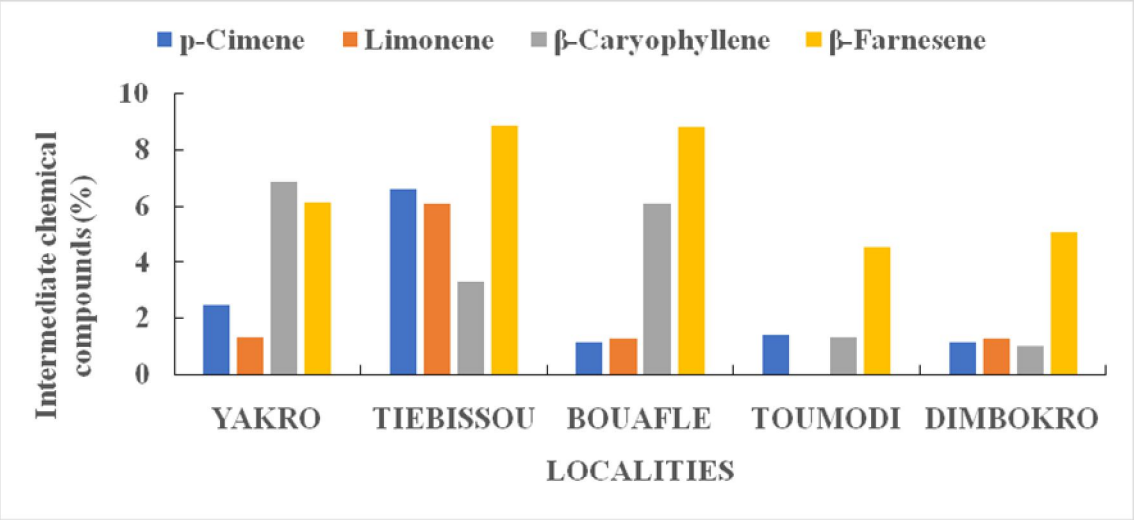

Figure 5: Distribution of intermediate chemical compounds in the essential oil of *Lippia multiflora* leaves according to locality

3.2 Antioxidant activities of *Lippia Multiflora* essential oils from the five locations studied

3.2.1 Trapping of the free radical 2,2-Diphenyl-1-picrylhydrazyl

Figure 6 shows the results of the antioxidant activity determined by the scavenging of DPPH free radicals and expressed by the 50% inhibition concentration of DPPH (IC_{50}).

The IC_{50} is inversely proportional to the antioxidant activity of a substance. This parameter expresses the quantity of antioxidant required to reduce the concentration of the free radical by 50%. The lower its value, the higher is the antioxidant activity of the substance **(Khoudali*et al.*, 2014)**.

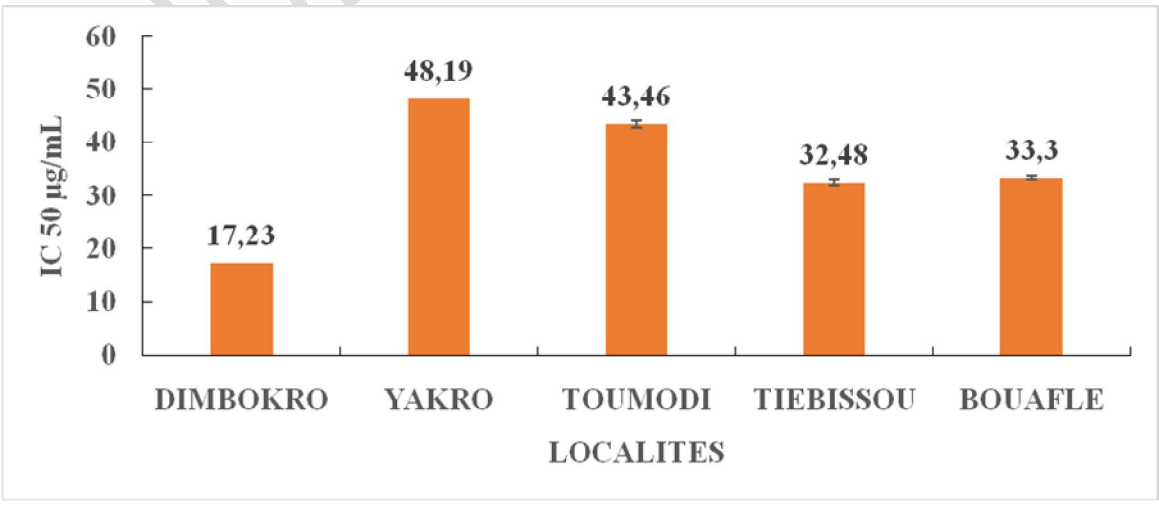

Figure 6: Inhibition concentration (IC_{50}) of DPPH trapping as a function of locality

3.2.2 Trapping of the free radical 2,2-azino-bis-(3-ethylbenzothialozine-6-sulphonic acid)

Figures 7 and 8 show, respectively, the calibration curve for Trolox (ABTS) over a range of concentrations and the variation in antioxidant activity of the different essential oil samples studied, expressed as a percentage of ABTS inhibition, as a function of locality.

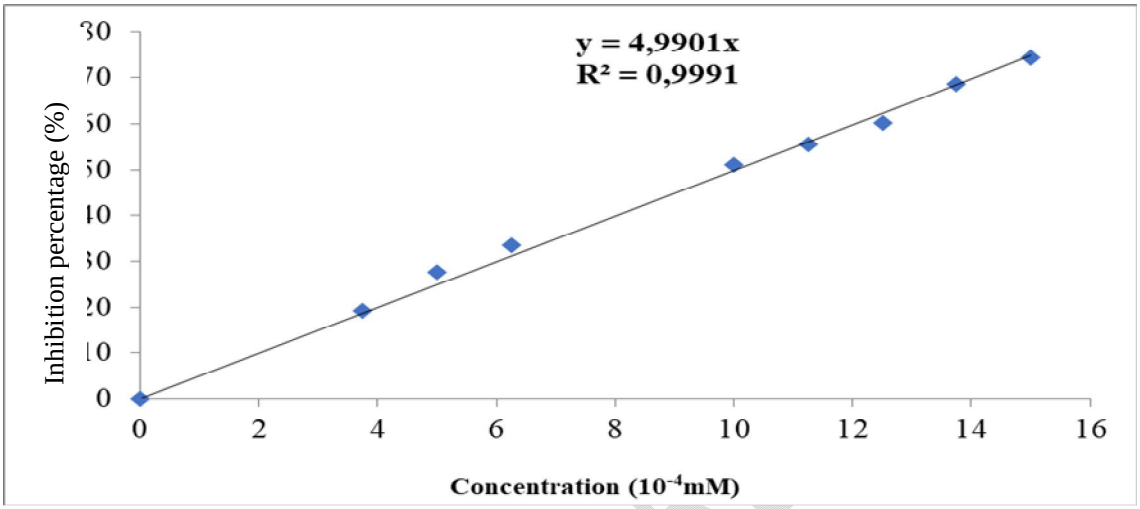

Figure 7 : Trolox calibration (ABTS)

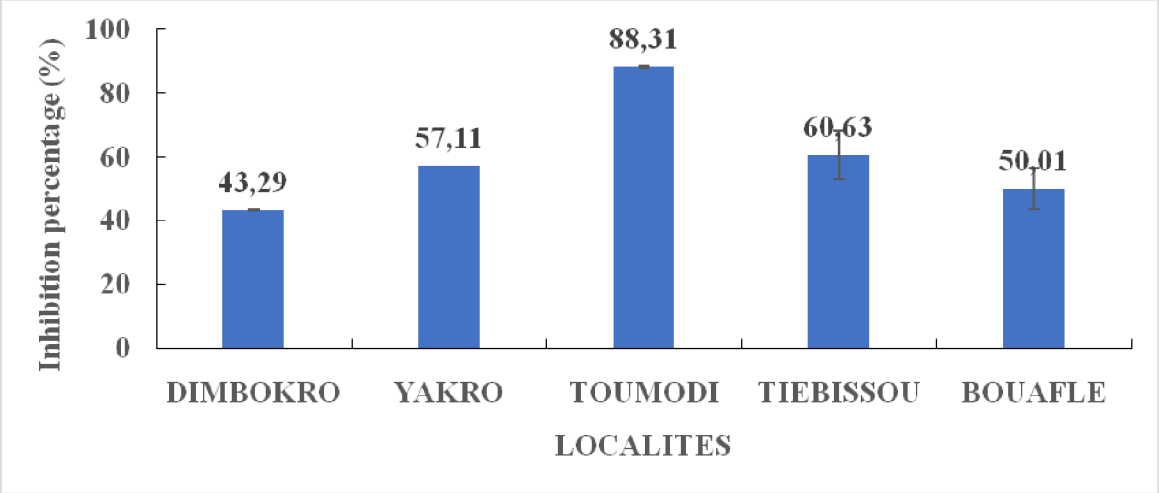

Figure 8: Inhibitionpercentage (%) of ABTS according to locality

3.3DISCUSSIONS

3.3.1 Chemical composition of different oils according to locality

The main chemical compounds that characterize*Lippia multiflora* essential oil are present in samples from five (5) localities **(Table 1)**. According to figures 4 and 5, trans-geraniol is dominant in the localities of DIMBOKRO (49.43%) and TOUMODI (47.82%), while it is weakly represented in YAKRO (0.08%) and TIEBISSOU (0.6%). α-citral is most abundant in TIEBISSOU (21.87%), TOUMODI (17.20%) and DIMBOKRO (16.38%), while it's moderately represented in BOUAFLE

(12.10%) and only slightly present in YAKRO (9.43%). Linalool is in the majority in BOUAFLE (19.99%) and in the minority in YAKRO (9.56%), TOUMODI (4.05%), DIMBOKRO (2.89%) and TIEBISSOU (2.22%). Germacrene D is predominant in YAKRO (11.02%) while it is weakly represented in BOUAFLE (5.27%), TIEBISSOU (4.06%), DIMBOKRO (2.93%) and TOUMODI (2.43%). The *α*-phellandrene is more abundant in TIEBISSOU (12.79%) and less abundant in YAKRO (3.61%), BOUAFLE (3.21%), DIMBOKRO (2.83%) and TOUMODI (1.66%). *β*-citral is more abundant in TIEBISSOU (16.74%), BOUAFLE (9.44%) and moderately present in TOUMODI (11.59%) and DIMBOKRO (11.57%) and weakly present in YAKRO (6.89%). Eucalyptol is more abundant in YAKRO (13.91%) and only slightly present in TIEBISSOU (4.02%) and BOUAFLE (0.07%), but absent in TOUMODI and DIMBOKRO. (±)-Trans-geraniol is preponderant in BOUAFLE (18.68%) and weakly represented in YAKRO (6.00%), TOUMODI (2.31%), TIEBISSOU (1.80%) and DIMBOKRO (0.90%).

Generally speaking, the chemical composition of the essential oil of *Lippia multiflora* leaves differs from one locality to another. The essential oil from YAKRO is dominated by Eucalyptol (13.91%). That of BOUAFLE is dominated by Linalool (19.99%), (±)-Trans-nerolidol (18.68%) and α-citral (12.10%). That of TIEBISSOU is dominated by *α*-citral (21.87%), *β*-citral (16.74%) and *α*-phellandrene (12.79%). The essential oil samples from TOUMODI and DIMBOKRO are dominated by *trans*-geraniol (47.82% / 49.43%),*α*-citral (17.20% / 16.38%) and *β*-citral (11.59% / 11.57%). The latter two localities appear to have a similar essential oil profile. This study shows great variability in the chemical composition of the essential oil of Lippia multiflora leaves. Such variability had already been observed by **Soro *et al.* (2015)** for the essential oil of *Lippia multiflora* leaves harvested in the localities of Abidjan, Toumodi and Bondoukou. The essential oils from TOUMODI and DIMBOKRO are similar to those from Abidjan, with the exception of Thymol (6.6%), which is only slightly present in the essential oil from Abidjan and absent from our last two localities. The results obtained for the TOUMODI locality in this study are almost similar to those obtained by Soro et al. (2015) for the same locality.Furthermore, the results from the YAKRO locality differ from that obtained by **Tia *et al.* (2013)** for the same locality where three majority compounds that are Eucalyptol (11.4%), Linalool (22.3%) and α-citral (10.6%) were identified while Eucalyptol (13,91%), Germacrene D (11.02%), Linalool (9.56%) and α-citral (9.43%), β-citral (6.89%) and β-farnesene (6.00%) are present in our sample. The majority compounds present in the essential oil of *Lippia multiflora* leaves in this study have already been identified by Kanko et al in samples from twelve (12) regions of Côte d'Ivoire **(Kanko et al., 2010)**. Similarly, the major compounds such as Germacrene D, trans-geraniol and (±) -trans-nerolidol from their study are present in our samples. However, the major compounds in our samples of *Lippia multiflora* leaves essential oil are absent from those from Burkina Faso and Benin with the exception of Germacrene D and Linalool respectively **(Tia, 2012)**.

The chemical compositions of *Lippia multiflora* essential oil from the five (5) localities in the study and those in the literature showed the existence of a large number of chemotypes in the West African

region including Nigeria where three major compounds: Eucalyptol (60.5%), Sabinene (16.9%) and α-terpineol (14.1%) were obtained **(Tia, 2012)**.Taken together, the results obtained and those from the literature show that the chemical composition of *Lippiamultiflora*essential oil is largely influenced by the harvesting area. Indeed, weeds could influence the chemical composition of the oil **(Rao *et al.*, 2002 and Boukhatem *et al.*, 2010)**.

3.3.2 Antioxidant activities of *Lippia Multiflora* essential oils from the five localities studied

Figure 6 shows that the 50% DPPH inhibition concentrations (IC_{50}) of the essential oil of *Lippia multiflora* vary according to locality. The highest inhibition concentration (48.19 ± 0.01 µg/mL) was obtained by the locality of YAKRO and the lowest inhibition concentration (17.23 ± 0.01 µg/mL) came from the locality of DIMBOKRO, which has the highest antioxidant activity. These values are higher than those obtained by **Kobenan *et al.* (2022)** on the same plant with an IC_{50} value of 6.545 µg/mL. However, the essential oil of *Lippia multiflora* leaves from DIMBOKRO showed greater activity in the DPPH test.

The antioxidant activity of the essential oil samples from the different localities could be linked to the presence of the major compounds which are variously distributed in these samples **(Ruberto and Barata, 2000)**. Indeed, the YAKRO locality is mainly dominated by Eucalyptol and Germacrene D whereas the TOUMODI locality is dominated by trans-geraniol, *α*-citral and *β*-citral. The high activity of the DIMBOKRO sample would be due to trans-geraniol and trans-nerolidol. Thus, trans-geraniol and trans-nerolidol would have a higher DPPH radical reduction capacity than the other major compounds. In addition, the IC_{50} value of vitamin C, taken as a reference, being 44.98 µg/mL, the essential oil from DIMBOKRO, TIEBISSOU and BOUAFLE would have a higher antioxidant activity than vitamin C on the DPPH.

The results obtained by the ABTS test showed that the percentages of inhibition of the essential oil of *Lippia multiflora* varied according to locality **(Figure 8)**. The highest inhibition percentage (88.3 ± 0.4%) was obtained for the TOUMODI locality and the lowest (43.3 ± 0.2%) came from the DIMBOKRO locality. Thus, trans-geraniol, *α*-citral and *β*-citral would have a greater ABTS cation reduction potential than the other majorconstituents.The percentage of inhibition of *Lippiamultiflora*leaves essential oil from the different localities is higher than that obtained by **Kobenan et al. (2022)** on the same plant whose percentage of inhibition is 54.56%, except for the essential oil from the localities of DIMBOKRO and BOUAFLE. The percentage inhibition value for Trolox, taken as a reference, is 74.41%.Thus, the essential oil from the TOUMODI locality has a higher antioxidant activity than Trolox. All the essential oil samples tested showed antioxidant activity, which was reflected in their ability to reduce the free radicals DPPH and ABTS. This ability to reduce the free radicals that cause oxidation confirms the traditional use of these plants to treat certain oxidative diseases such as cancer, acute respiratory distress syndrome, diabetes, Alzheimer's disease and cardiovascular disease **(Sergeant *et al.*, 1998; Mates *et al.*, 2000)**. However, for most of

the essential oil samples tested, the order of activity obtained using the DPPH method did not correlate with that obtained using the ABTS method, with the exception of essential oil from the locality of TIEBISSOU. The results differed from one locality to another and from one method to another **(Del Castillo *et al.*, 2002)**. These variations could be explained by the mechanisms involved in the radical reduction reactions **(Amarti *et al.*, 2013; Ruberto and Barata, 2000)**. In addition, the essential oil samples were solubilized in methanol for the DPPH test and in ethanol for the ABTS test. The solubility of essential oils in these different solvents could explain the variations in activity between the two methods and influence their results **(Soares *et al.*, 2003; Yu *et al.*, 2002)**.

4. CONCLUSION

This study, carried out with the aim of assessing the variability of the chemical composition and antioxidant activity of the essential oil of *Lippia multiflora* leaves harvested in five localities in Côte d'Ivoire, showed that the chemical compositions and antioxidant activities vary from one locality to another. The analysis revealed that these oils are dominated by oxygenated monoterpenes, and identified eight major compounds including α-phellandrene, Eucalyptol (1,8-cineole), Linalool, *β*-citral (Neral), *trans*-geraniol, *α*-citral (Geranial), Germacrene D and (±)-trans-nerolidol.

The essential oil of *Lippia multiflora* leaves was found to have high trans-geraniol content in TOUMODI and DIMBOKRO, with proportions of 47.82% and 49.43% respectively. The essential oil samples from DIMBOKRO and TOUMODI showed higher antioxidant activities than ascorbic acid and Trolox in the DPPH and ABTS tests respectively. The high proportion of oxygenated monoterpenes, in particular *trans*-geraniol, seems to be responsible for these antioxidant activities. The results suggest that the essential oil of *Lippia multiflora* leaves could be a viable alternative to synthetic antioxidants, with potential applications in the food and pharmaceutical industries. Consequently, the development of this plant essence represents a crucial challenge for the management of plant resources.

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