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Effect of drying methods on the chemical composition of essential oils of *Xylopia aethiopica*fruits (Dunal) A. Richard (Annonaceae) from southern Senegal

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Abstract
The objective of this work is to study the variability of the chemical composition of essential oils of fruits of *Xylopia aethiopica* from southern Senegal. Essential oils were obtained by steam distillation on the fresh (F), shade-dried (DSh) and sun-dried (DS) fruits of *Xylopia aethiopica*. Analyzes of these essential oils carried out by GC/FID and GC/MS revealed three major compounds: β-pinene, 1,8-cineole and α-pinene in variable proportions. β-Pinene was identified for 29.9% (F), 15.1 to 31.2% (DSh) and 27.0 to 30.7% (SS), 1,8-cineole represented 14.7% (F), 14.5 to 15.1% (DSh) and 17.4 to 21.2% (SS) and α-pinene constituted 10.0% (F) 3.7 to 11,0% (DSh) and 6.6 to 10.0% (DS).

Keywords: *Xylopia aethiopica*, essential oils, chemical composition, β-pinene, pinene, 1,8-cineole

1. Introduction

Belonging to the Annonaceae family, *Xylopiaaethiopica* is a well known and widespread species in dense forests of Africa[1]. The essential oils of its organs (fruits, seeds, roots and barks) give it multiple uses in traditional and modern medicine. In tropical Africa, the air dried fruits of *Xylopiaaethiopica*are used for its nutritional and therapeutic properties. In Senegal, *Xylopia aethiopica* fruits powder is added as flavoring in some culinary preparations such as coffee and tea, in particular. In traditional medicine, it is effective against tropical diseases [2]. *Xylopia aethiopica* is also used to treat the following pathologies: conjunctivitis, bronchitis, dysentery and sore dents.In Nigeria, Asekun and Adeniyi (2004) [3] showed the effectiveness of the powdered root of *Xylopia aethiopica* cancer treatment.

The chemical composition of esssential oils of *Xylopia aethiopica* vary according to different factors including the development stage of the vegetative cycle, geographical area and the harvest period of the plant [4]. Several studies have focused on the qualitative and quantitative study of *Xylopia aethiopica*essential oils. They include among others those of Frederick *et al* (1996) [5]that reported sabinene (36.0%), 1,8-cineole (12.8%), linalool (3.9%), and terpinen-4-ol (7.0%); Bakary *et al*. (2003) [6] identified β-pinene (19.1%), γ-terpinene (14.7%), *trans*-pinocarvéol (8.6%) and *para*-cymene (7.3%) as major compounds; Koffi *et al*. 2008 [7] obtained β-pinene (23.6%) of α-pinene (11%) of sabinene (9.8%), germacrène D (8.3%) and 1,8-cineole (8.2%). Thus, it is reported in the literature that essential oils from dried fruits of *Xylopia* are mainly constituted by hydrocarbon compounds [8]. However, a significant decrease of hydrocarbon derivatives was noted with shade and sun dried fruits [8]. Insecticidal activity of essential oils of fruit *Xylopia aethiopica* against *Callosobruchus maculatus* was obtained by Koffi *et al*. (2012) [9]. Other studies have shown good antibacterial properties, miticidal and cytotoxical*Xylopia aethiopica*essential oils [10-13]. In our knowledge, the chemical composition of essential oils of *Xylopia aethiopica*fruits has not been studied in Senegal.In this work, we propose to study the variability of the chemical composition of essential oils of *Xylopia aethiopica* fruits collected in Senegal according to the drying methods. We will also study the oils on chiral column to determine the enantiomer that are present for each identified chiral molecules. This could allow the valorization of essential oils of *Xylopia aethiopica*from Senegal.

2. Materials and methods

2.1 Plant Material

*Xylopia aethiopica*fruitswere collected in southern Senegal in June 2015. A voucher specimen was deposited in the herbarium of the "*Institut Fondamental d'Afrique Noire de l'Université Cheikh Anta Diop, University of Dakar*". Fruits have the form of small beans with a length of 4 cm. The sample was divided into three portions: the first was kept fresh, the second was driedunder the laboratory conditionsin the shade of the sun and the third was sun dried.

2.2 Extraction of essential oils

The oils were extracted from fresh fruits, shade dried and sun dried fruits for 5, 10, 15 and 20 days. *Xylopia aethiopica* oils were distilled by steam distillation with 250g for 90 min using a Clevenger type apparatus. Essential oils thus obtained were stored in amber vials and stored at 4°C before use.

2.3 Analysis of essential oils by chromatographic methods

In GC/FID as in GC/MS, the temperature conditions were as follows: it is initially maintained at 40 °C for 5 mn, after the temperature undergoes a gradual increase of 8 °C/mn up to the limit of 280 °C where it is maintained for 5 mn.The injector works in splitless mode at 280 °C with a split flow of 30 ml/mn. Detector temperature is 290 °C. Helium is the carrier gas with a constant flow of 1.5 ml/mn. The capillary column used was Optima-5-accent type, 5% phenylmethylsiloxane: 30 m x 0.25 mm i.d., 0.25 µm film thickness (Macherey-Nagel, Germany). The volume of sample injected for each analysis was 1 µl (10mg/10ml *n*-hexane). The air flow and hydrogen were respectively 350 and 35 ml/mn in both GC/FID and GC/MS.

GC/FID: The device is a Trace GC Ultra (Thermo Electron Corporation, Interscience, Milan, Italy) coupled with a flame ionization detector.

GC/MS: The Agilent type of mass spectrometer technology 5973 Network Mass Selective Detector Quadrupole was associated to a gas chromatograph, Agilent Technologies 6890N (G1530N), USA. The relative abundance of the peaks of the spectra was between 50 and 550 m/z, with an ionization energy of 70 eV.The identification of compounds of *Xylopia aethiopica*essential oils was performed by comparing the mass spectra obtained with those of the computerized database (Wiley 275 L) and retention indices with those given in the literature [14, 15]. Theidentification of compounds was completed by injection of pure reference molecules.

3. Results and Discussion

3.1 Results

The extraction of dried fruits of *Xylopia aethiopica* by steam distillation gived a pale yellow essential oils with yields of 1.9% (F); 0.8, 1.2, 1.3 and 1.2% (DSh); 1.2, 0.9, 1.0 and 1.1% (DS) after 5, 10, 15 and 20 days of drying, respectively.The results of analysis of the essential oils from fresh and shade-dried fruits of *Xylopia aethiopica* by GC/FID and GC/MS are showed in table 1.

Table 1: Chemical composition of essential oils from fresh and shade-dried fruits of*Xylopia aethiopica*

Compounds	Retention indices	Fresh fruits (F)	Shade-dried fruits (DSh)			
			5th day	10th day	15th day	20th day
α-Pinene	937	10.0	3.7	8.3	9.9	11.0
Sabinene	976	6.8	2.6	4.2	4.9	5.0
β-Pinene	982	29.9	15.1	25.5	29.2	31.2
Myrcene	989	0.8	-	0.4	0.4	-
Not identified	993	0.3	-	0.2	0.3	-
Not identified	997	0.5	-	0.3	0.3	-
α-Phellandrene	1008	2.5	-	0.6	0.5	0.4
α-Terpinene	1020	0.3	-	0.2	-	-
para-Cymene	1028	3.1	3.1	2.6	2.9	2.9
Limonene	1033	1.6	1.0	1.0	1.2	1.2
1,8-Cineole	1037	14.7	14.5	14.9	15.0	15.1
γ-Terpinene	1062	0.3	-	0.3	-	-
cis-Sabinene hydrate	1075	0.2	0.7	0.9	0.6	0.7
Linalool	1100	2.8	5.9	3.7	3.1	2.9
n-Nonanal	1105	-	0.5	0.4	-	0.3
α-Campholenal	1133	-	-	0.3	0.3	-
trans-Pinocarveol	1150	1.9	6.3	4.8	4.5	4.4
5-Undecyne	1165	-	-	0.2	-	-
Pinocarvone	1171	0.6	0.8	1.1	1.2	1.0
δ-Terpineol	1176		0.5	0.4	0.3	-
Terpinen-4-ol	1187	0.9	2.0	1.2	1.2	1.2
Cryptone	1195	0.3	0.7	0.7	0.6	0.5
α-Terpineol	1200	1.5	4.6	2.6	2.1	1.9
Myrtenol	1203	1.7	5.4	4.1	4.0	3.9
α-Phellandrene epoxide	1210	-	-	0.3		-
Verbenone	1216	-	0.7	0.5	0.4	-
trans-Carveol	1224	-	0.4	0.3	-	-
Carvone	1251	-	0.4	0.3	-	-
δ-Elemene	1345	1.1	0.7	0.5	0.4	0.4
α-Cubebene	1358	-	0.5	0.3	0.3	0.3
α-Copaene	1390	2.9	5.6	3.1	3.2	3.1
β-Cubebene	1400	0.6	1.1	0.7	0.7	0.7
Cyperene	1423	0.7	1.5	0.8	0.9	0.9

(*E*)-β-Caryophyllene	1434	3.3	0.7	2.5	1.6	1.3
trans-α-Bergamotene	1440	0.6	-	-	-	-
γ-Elemene	1445	2.0	0.6	1.3	1.1	0.9
Aromadendrene	1460	0.8	0.5	0.6	0.4	0.3
α-Humulene	1467	1.0	0.7	0.6	0.5	-
α-Amorphene	1486	-	-	0.3	-	-
Germacrene D	1496	2.3	-	1.5	1.2	1.2
β-Selinene	1500	-	-	-	-	-
α-Muurolene	1508	0.5	1.9	1.0	0.8	0.8
γ-Cadinene	1516	0.7	-	0.5	-	0.3
δ-Cadinene	1529	0.5	1.3	0.7	0.8	0.8
cis-Calamenene	1534	-	1.2	0.5	0.3	0.3
Elemol	1568	-	1.1	0.4	0.5	0.5
Spathulenol	1595	0.5	2.0	0.8	0.8	0.8
Caryophyllene oxide	1600	-	0.4	-	-	-
Not identified	1603	-	0.8	-	-	-
Salvial-4(14)-en-1-one	1613	-	0.8	0.3	0.3	0.3
Not identified	1631	-	1.2	0.3	0.4	0.4
γ-Eudesmol	1642	1.8	7.2	2.5	2.6	2.7
β-Eudesmol	1670	-	0.4	0.2	-	-
α-Eudesmol	1676	-	0.9	0.3	0.3	0.4
Monoterpenic hydrocarbons		55.4	25.2	43.1	48.9	51.8
Oxygenated monoterpenes		24.6	43.4	36.2	33.6	31.7
Sesquiterpenic hydrocarbons		17.1	16.5	15.2	12.3	11.5
Oxygenated sesquiterpenes		2.2	13.5	4.6	4.5	4.6
Not identified		0.7	1.4	0.9	0.7	0.4

Table 1 reveals the presence of 33 (F) and 50 (DSh) volatile compounds for fresh and shade-dried materials, corresponding to 99.3% and 98.6 to 99.6% of the total oils content, respectively. The oils of the fresh material were composed of 80.0% of monoterpenes which 55.4 and 24.6% of hydrocarbon and oxygenated compounds, respectively. The major compounds identified in these essential oils were monoterpenic hydrocarbons such as β-pinene, α-pinene and sabinene that constituted 29.9, 10.0 and 6.8%, respectively of the total oils content. The oxygenated monoterpenes were dominated by 1,8-cineole (14.7%). Other compounds which representated less than 5.0% levels were also obtained in oils from fresh fruits such as *para*-cymene (3.1%), linalool (2.8%), *trans*-pinocarveol (1.9%), myrtenol (1.7%) and α-terpineol (1.5%). Sesquiterpenes represented 19.2% with 17.1% of hydrocarbonic compounds. The major sesquiterpenes were α-copaene (2.9%), (*E*)-β-caryophyllene (3.3%), germacrene D (2.3%) and γ-eudesmol (1.7%).Oils extracted from shade-dried fruits were rich in monoterpenes: 68.6, 79.2, 82.3 and 83.4% respectively after 5, 10, 15 and 20 days of drying. The major compounds identified in these oils were β-pinene (15.1, 25.5, 29.2 and 31.2%), 1,8-cineole (14.5, 14.9, 15.0 and 15.1%), α-pinene (3.7, 8.3, 9.9 and 11.0%) and sabinene (2.6, 4.2, 4.9 and 5.0%). Other representative compounds identified in the oils were: *trans*-pinocarveol (6.3, 4.7, 4.5 and 4.4%), myrtenol (5.4, 4.1, 4.0 and 3.9%), linalool (5.9, 3.7, 3.1 and 2.9%), *para*-cymene (3.1, 2.6, 2.9 and 2.9%) and α-terpineol (4.6, 2.6, 2.1 and 1.9%). Sesquiterpenes constituted 30.0, 19.8, 16.8 and 16.1%, respectively after 5, 10, 15 and 20 days of drying. They were mainly dominated by α-copaene (5.6, 3.1, 3.2 and 3.1%), γ-eudesmol (7.2, 2.5, 2.6 and 2.7%) and (*E*)-β-caryophyllene (0.7, 2.5, 1.6 and 1.3%). The results obtained on the oils from sun-dried fruits are showed in Table 2.

Table 2: Chemical composition of essential oils from sun-dried fruits of *Xylopia aethiopica*

Compounds	**Retention indices**	**Sun dried fruits (DS)**			
		5th day	**10th day**	**15th day**	**20th day**
α-Pinene	937	9.7	6.6	9.6	10.0
Sabinene	976	5.2	3.9	4.3	3.9
β-Pinene	982	30.6	27.0	30.7	30.3
Myrcene	989	0.4	-	-	-
para-Cymene	1028	4.3	4.9	4.5	4.3
Limonene	1033	1.5	1.6	1.5	1.3
1,8-Cineole	1037	17.7	21.2	18.9	17.4
cis-Sabinene hydrate	1075	0.4	0.5	-	
Linalool	1100	3.4	3.2	3.1	2.5
n-Nonanal	1105	0.4	-	-	0.3
α-Campholenal	1133	-	-	-	0.4
trans-Pinocarveol	1150	4.7	5.6	6.0	5.7
5-Undecyne	1165	-	-	-	0.3
Pinocarvone	1171	1.3	1.6	1.3	1.2
Terpinen-4-ol	1187	1.5	1.8	1.7	2.2
Cryptone	1195	-	-	-	-
α-Terpineol	1200	0.5	-	-	0.7
Myrtenol	1203	2.1	2.0	2.0	1.8
Verbenone	1216	4.5	5.8	5.9	6.1

Carvone	1251	0.4	-	0.6	0.7
trans-2-Decenal	1262	0.3	-	-	0.4
δ-Elemene	1345	-	0.4	-	0.4
α-Cubebene	1358	-		-	0.4
α-Copaene	1390	-	0.4	-	-
β-Cubebene	1400	3.0	3.6	3.0	2.7
Cyperene	1423	0.6	0.5	0.5	0.4
α-Muurolene	1508	0.6	1.0	0.8	0.8
δ-Cadinene	1529	0.8	1.0	-	0.6
cis-Calamenene	1534	0.9	0.8	0.7	0.6
Elemol	1568	0.8	0.4	0.5	0.4
Spathulenol	1595	0.6	0.7	0.5	0.5
Caryophyllene oxide	1600	0.8	0.9	0.7	0.6
Salvial-4(14)-en-1-one	1613	-	-	-	0.4
Not identified	1631	-	0.4	0.4	0.4
γ-Eudesmol	1642	0.5	0.6	0.5	0.4
α-Eudesmol	1676	2.5	3.2	2.4	2.1
Monoterpenic hydrocarbons		41.9	37.3	41.1	40.1
Oxygenated monoterpenes		37.5	42.1	39.5	39.7
Sesquiterpenic hydrocarbons		6.8	7.7	5.5	5.9
Oxygenated sesquiterpenes		4.6	6.2	4.4	4.4
Not identified		0.5	0.6	0.5	0.4

A total of 35 compounds were identified in oils extracted from sun-dried fruits representing 99.4 to 99.6% of the total oil content. They showed a significant difference of 15 compounds with oils from the shade-dried fruits. Monoterpenes were the main constituents identified in these oils. They represented 79.4, 79.4, 80.6 and 79.8% after 5, 10, 15 and 20 days of drying in the sun respectively. They were characterized by β-pinene (30.6, 27.0, 30.7and 30.3%), 1,8-cineole (17.7, 21.2, 18.9 and 17.4%), α-pinene (9.7, 6.6, 9.6 and 10.0%), myrtenol (4.5, 5.8, 5.9 and 6.1%), *trans*-pinocarveol (4.7, 5.6, 6.0 and 5.7%), *para*-cymene (4.3, 4.9, 4.5 and 4.3%), sabinene (5.2, 3.9, 4.3 and 3.9%), linalool (3.4, 3.2, 3.1 and 2.5%) and α-terpineol (2.1, 2.0, 2.0 and 1.8%). Sesquiterpenes represented 11.4, 13.9, 9.9 and 10.3% and the most important were β-cubebene (3.0, 3.6, 3.0 and 2.8%) and γ-eudesmol (2.5, 3.2, 2.4 and 2.1%).

3.2 Discussion

The results showed that drying has significant influences on the yield and chemical composition of essential oils*Xylopia aethiopica*. The most important yield was obtained with the fresh material. This may be explained by the fact that during drying, volatile compounds contained in aromatic plants can be evaporate and the yield decrease. Variations notedin the different groups of constituents according to the drying are showed in Fig.1.

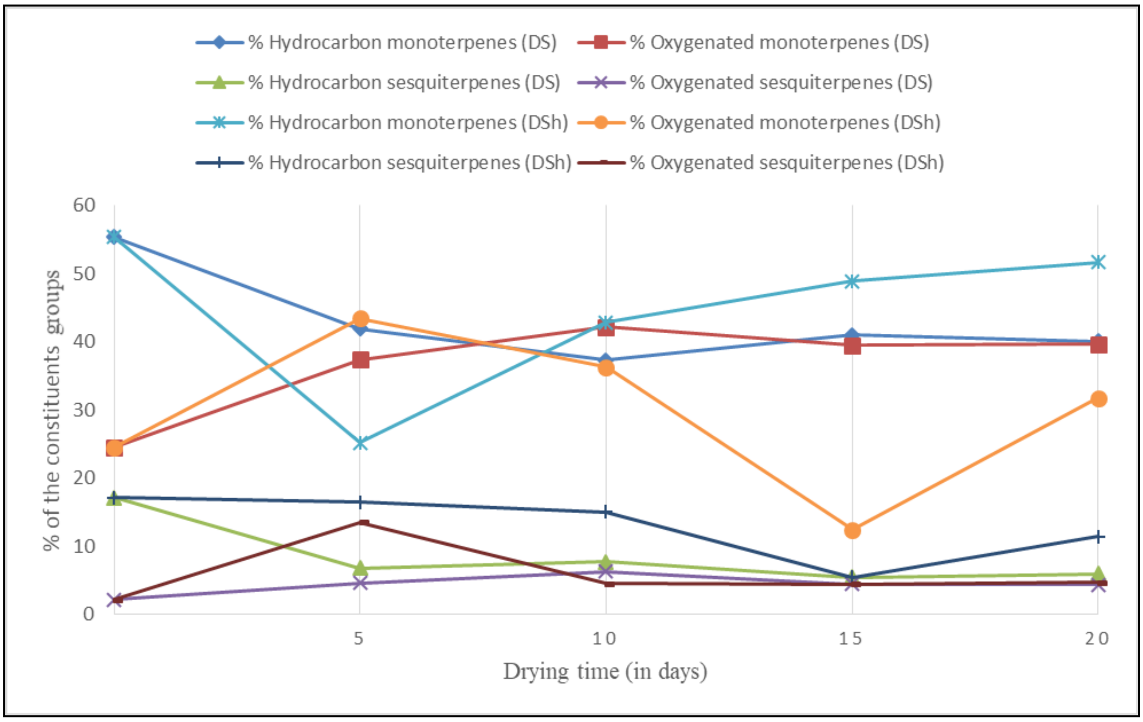

Fig 1: Evolution of terpenic groups from essential oils of*Xylopia aethiopica*according to the drying

Fig. 1 shows that fresh fruits contain more monoterpenic hydrocarbons (55.4%) and less oxygenated monoterpenes (24.6%) than dried fruits and this, whatever the nature and drying duration. Highest oxygenated monoterpenes rates (43.4%) was obtained after 5 days of drying in the shade. Beyond the fifth day, the sun-dried fruits showed more oxygenated monoterpenes with a maximum of 42.2% at the 10th day. Lowest monoterpenichydrocarbons rates were obtained after 5th day (25.2%) and 10th day (37.3%) of drying in the shade and the sun, respectively. These levels increased with the drying time until the 20th day with 51.7% (DSh) and 40.1% (DS). Fig.1 also revealed more important evolution of oxygenated sesquiterpenes rate for drying in the shade thanin the sun. This rate peaked (13.5%) on the 5th day of drying and decreased until 10th day (4.5%) and then remained constant until the 20th day. Meanwhile, the content of oxygenated sesquiterpenes for sun dried was highest (6.2%) at 10th day and then decreased at 20th day until 4.4%. The results showed that during the drying process, sesquiterpenichydrocarbons were more abundant after shade-dried and were maximum for fresh material (17.1%). Drying also affected the content of the major compounds of oils (Fig.2).

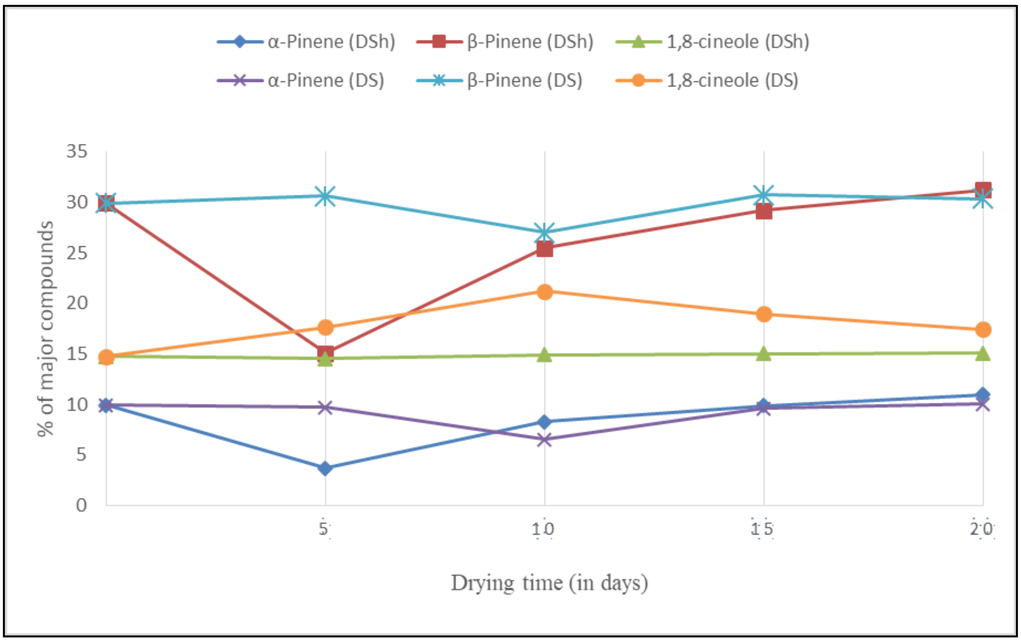

Fig 2: Evolution of major compounds from essential oilsof*Xylopia aethiopica*according to the drying

From this figure, it appears that β-pinene is the major constituent of *Xylopia aethiopica*oils except for the 5th day of the drying in the shade. It is followed by 1,8-cineole which remains constant throughout the drying in the shade with a rate about 15.0%. However, its content is more important for sun-dried fruits than other oils, it reaches a maximum (21.2%) at the 10th day. α-Pinene has showed the highest rate after 20 days ofdrying in the shade (11.0%) and the sun (10.0%). Whatever the nature of drying the three major compounds constitute the major overwhelmingof the essential oils: 54.6% for fresh material; 33.3, 48.7, 54.1 and 57.3% (DSh), 57.9, 54.7, 59.3 and 57.8% (DS) after 5, 10, 15 and 20 days of drying, respectively. The present results showed variations on the concentration of volatile compounds due to the drying, this fact was observed by Mary *et al*. (2012) [8]. The study also revealed that the rates of major compounds were more higher after sun than shadedrying.It should be noted that the chemical composition of *Xylopia aethiopica*essential oils from Senegal is near of the species from Togo, reported by Koffi *et al*. (2008) [7]. These authors mainly obtained β-pinene (23.6%), α-pinene (11.0%), sabinene (9.8%), germacrene D (8.3%) and 1,8-cineole (8.2%). However, it differs of those from Nigeria described by Asekun and Adeniyi (2004) [3] which contained 1,8-cineole (15.2%), sabinène (6.6%) and terpinen-4-ol (4.1%) as major compounds. In addition, Senegalese oils remain significantly different of the sample analyzed in Mali by Bakary *et al*. (2003) [6]. The latter reported the presence of β-pinene (19.1%), γ-terpinene (14.7%), *trans*-pinocarveol (8.6%) andthe *para*-cymene (7.3%). It is noted that drying strongly influences the composition of essential oils. To this is added other factors such as the locality of harvest or chemical reactions which may occur during distillation. For this purpose, oxygenated and hydrocarbon monoterpenes (mono and bicyclic), and oxygenated sesquiterpenes are the most vulnerable to structural modifications [16]. The presence of a significant amount of γ-eudesmol in oils from Senegal 1.7% (F) 2.5 to 7.2% (DSh) and 2.1 to 3.2% (DS) shows a particular character of these essential oils. In our knowledge, eudesmol was only reported with a significant amount by Véronique and Josy (1997) [17] in *Xylopia aethiopica* essential oils from Benin.

4. Conclusion

In this work, it is identified 50 compounds from different samples of oils. Effect of drying on the chemical composition of the oils and the evolution of the different groups of compounds and major constituents were studied. Three major compounds were identified: β-pinene, 1,8-cineole and α-pinene inthe fresh, shade-dried and sun-dried fruits.1,8-Cineole is known for its antifungal, anti-infectious and bactericidal properties. This study showed that its rate increases considerably with the effect of drying. It goes from 14.7, 15.1 and 21.2% for the fresh plant, after 20 days of drying in the shade and 10 days of drying in the sun respectively. Variations were also noted with drying for the other volatiles compounds. It appears from our work that drying would be a good alternative for the correction of certain oils and in particular essential oil of *Xylopia aethiopica*.

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