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Effect of Drying the Chemical Composition of the Essential Oil of *Ocimum gratissimum* L (Lamiaceae) Harvested in the Tonkpi Region (Ivory Coast)

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Abstract

In this study, we were interested in the valuation of *Ocimum gratissimum*, an aromatic and medicinal plant of the Ivorian flora. The essential oils of the leafy branches of *Ocimum gratissimum* were extracted by hydrodistillation with a Clévenger-type device. The yield of the oils obtained increases from (0.05±0.01%) to (0.42±0.08%) depending on the drying time. The density of essential oils is almost constant at 0.80. The chemical composition of essential oils was identified after analysis of chromatograms and mass spectra. The number of phytocompounds also increases with the number of drying days from 32 to 37. These compounds are marked by the presence of hydrocarbon monoterpenes from (29.11% to 49.1%), and oxygenated from (40.33% to 50.02%) and the presence of hydrocarbon sesquiterpenes from (5.52% to 8.55%), and oxygenated from (0.80% to 2.16%). The number of major compounds is 5 on day 0 and decreases to 4 on the other days, and have the following proportions: thymol from (33.60% to 44.73%), β-caryophyllene from (2.54% to 3.29%), para-cymene (12.32% to 25.60%), γ-terpinene (10.09% to 11.41%), Bis(2-ethylhexyl)phthalate (12.12% to 0.42%). bis (2-ethylhexyl) phthalate which is in the minority during the other drying days.

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Keywords and phrases: *Ocimum gratissimum*; essential oil; effect of drying; yield; chemical composition.

1. Introduction

Plants have long time presented a very important role for humanity, as they can synthesize a large number of complex organic molecules often endowed with potential biological activities. They are traditionally used for healing, feeling well, flavoring food and preserving food [1]. The African continent is endowed with a very rich biodiversity with many plants used as natural food and for therapeutic purposes. The side effects of synthetic molecules and economic constraints have in recent years led to the use of medicinal plants for therapeutic purposes [2]. Thus, for economic and socio-cultural reasons, more than 80% of African populations use traditional medicine [3], the prerogative of poor people [4]. The knowledge of essential oils has had its existence for a long time since the prehistoric man who has already practiced, in his own way, the extraction of fragrant principles from plants. Then gradually, essential oils were known for their therapeutic virtues and then have become common remedies in traditional medicine [5].

In an attempt to find new remedies for the current scourges, the scientific community is turning more and more to the constituents of essential oils; because a significant number of volatile compounds have shown remarkable pharmacological activities against diseases such as cancer [6]. Essential oils are therefore an interesting source of new compounds in the search for bioactive molecules. The therapeutic potential of essential oils from plants needs to be explored. This is why we were interested in *Ocimum gratissimum*, an aromatic species and essential oils used by the populations in traditional medicine. It is an annual herb widespread in tropical regions and also used by people as a spice [7].

Studies have been carried out on the chemical composition and pharmacological activity of the EO of the leaves of the species. In Ivory Coast, the essential oil extracted by hydrodistillation consists of monoterpenes (hydrocarbon and oxygenated) and hydrocarbon sesquiterpenes. The major compounds are p-cymene (37.79%) and thymol (24.757%). Essential oil (EO) has potential insecticidal activity [8, 9]. It is cytotoxic, antioxidant and antimicrobial [10, 11]. The major compounds are thymol (46.10%), γ-terpinene (17.56%) and p-cymene (7.53%) [11]. The main compounds of the EO of the leaves of the Togolese species are thymol (31.57%) and γ-terpinene (27.10%). EO has good antibacterial activity [12]. The EO of the pale yellow Vietnamese species has a yield of (0.23±0.07)%. It is composed of monoterpenes (hydrocarbon and oxygenated) and sesquiterpenes (hydrocarbon and oxygenated). The majority compound is eugenol

(65.13%) [13, 14]. That of the Nigerian species extracted by hydrodistillation has a yield of 0.92% [15]. The EO of the Brazilian species has a yield of 0.17%; the major constituent is eugenol (72.26%). It has good antibacterial activity [16]. Studies have also been done in India. The essential oil from aerial parts of *O. gratissimum* was extracted by hydro-distillation. The yield was 1.31%. The main fractions were classified as phenylpropene (55.73%), sesquiterpenes (27.49%) and monoterpenes (16.14%). The major constituents were eugenol (54.42%), germacrene D (15.43%), β-ocimene (12.37%), and caryophyllene (4.59%) [17]. The yield of the *O. gratissimum* essential oil varied between 0.12% and 1.66%. EO was predominantly accumulated phenylpropenes, (55.7% - 57.3%) followed by sesquiterpenes (27.5% - 38.1%), and monoterpenes (4.0% - 16.1%). Eugenol, germacrene-D, β-ocimene, 1,8-cineole, β-selinene, caryophyllene, γ-murolene, p-cymene, and thymol, are major constituents of OGEO from various origins [18]. To our knowledge, the effect of drying the chemical composition of the essential oil of *Ocimum gratissimum*, the Ivorian species, has never been studied.

In the present work, we shall contribute to the valuation of *O. Gratissimum*, a Lamiaceae used in traditional medicine. So, we have highlighted the effect of drying the yield of EO extracted by hydrodistillation and then elucidated the chemical composition by gas chromatography coupled with a mass spectrometer.

2. Materials and Methods

2.1. Material

The plant material consists of leafy branches of *Ocimum gratissimum*. The harvest was carried out on February 12, 2022 in the high school district in the city of Man. The city is located in the TONKPI region in the west of Côte d'Ivoire. The plant was identified and authenticated by a technician at the 'Center National de Floristique' (CNF) in Abidjan (Côte d'Ivoire) using the existing herbarium under number H UCJ 008879.

2.2. Methods

2.2.1. Drying

After the harvest, the plant biomass was dried in the laboratory at room temperature. The raw material is spread in thin layers and turned frequently. Leafy twigs were dried from the first day (day 0) to the seventh day (day 6). We made seven samples of plant material.

2.2.2. Extraction

The first extraction of EO is made fresh on the day of harvest, the second 24 hours later, and so on until the 7th day.

Extractions of essential oils from leafy twigs cut into small pieces were made by hydrodistillation operations using a Clevenger-type device for 2 hours. In a 6 L round-bottomed flask containing a quantity of water (2.5 L) and plant material, a cooler is mounted. The whole is brought to the boil with a heating cap. The water vapor carries the volatile products towards the condensation column. Condensed vapor is a binary azeotropic mixture composed of floral water and essential oil. The essential oil is separated from the water by decantation. It is dried over anhydrous sodium sulphate. The different EO samples are put in bottles covered with aluminum foil and stored in a refrigerator at -9°C [17,19].

Yield calculation

The yield (r) of extracted EO is calculated as follows [20]:

$$r = \frac{m_{EO}}{m_{MV}} \times 100$$

With: m_{EO}: mass of EO (g), m_{MV}: mass of plant material (g).

2.2.3. Determination of phytochemical composition

The analysis of EO diluted in dichloromethane (1:100) was carried out on a GC chromatograph (7890A, Agilent Technologies) coupled to a mass spectrometer (5975C, Agilent Technologies). A sample of HE (1µL) was injected into an HP-5MS capillary column at 250°C. The oven temperature was programmed at 40°C for 5 min, then at 2°C/min for 15 min up to 250°C, with a flow rate of 10°C/min up to 300°C. Helium was used as the carrier gas with a flow rate of 1 mL/min. The MS detector had a temperature of 280°C and a voltage of 1.4 kV. Only ions whose mass/charge ratio is between 40 and 500 can be detected. The identification of the compounds was carried out by comparing the retention indices, calculated from the retention times and the mass spectra obtained with those of National Institute of Standards and Technology (NIST) database and literature [21].

$$RI = 100\left[n + \frac{t_R(C_i) - t_r(C_n)}{t_r(C_{n+1}) - t_R(C_n)}\right]$$

RI or IR: retention index

C_i: Unknown compound of EO;

C_n: linear alkane (comprising n C atoms) whose retention time is just before that of the unknown EO compound;

C_{n+1}: Linear alkane (comprising n C atoms) whose retention time is just after that of the unknown compound;

n: carbon number of the linear alkane.

$t_R(C_n)$ retention time of the linear alkane with n carbon atoms.

3. Results and Discussion

3.1. Extraction result

3.1.1. Physical parameters of extracted EOs

The essential oil obtained has a pale yellow color with an aromatic smell.

The values of the various parameters are recorded in Table 1.

Table 1. Physical parameters of EO extracted during dry.

Drying time (days)	0	1	2	3	4	5	6
Organ mass (g)	400	400	500	500	500	500	500
Mass of EO (g)	0.2	0.2	1.4	1.47	2.1	2.1	2.1
EO volume (ml)	0.4	0.5	2	2.2	2.8	2.8	2.8
EO yield(%)	0.05±0.01	0.05±0.01	0.28±0.05	0.294±0.063	0.42±0.08	0.42±0.08	0.42±0.08
Density	0.775	0.776	0.820	0.780	0.777	0.777	0.777

EO: Essential oil; % : Percentage

Looking at Table 1, we can see that the density of the essential oil from day 0 to day 6 is almost constant. This observation could be explained by the fact that the density characterizes the essential oil. It is the same for a given oil.

3.1.2. Effect of drying HE yield

The amount of essential oil extracted increases from day 0 to day 4. It reaches a threshold from day 4.

Figure 1 shows that the essential oil yield remains constant from day 0 to day 1; this is explained by the moisture content of the fresh leafy twigs of *Ocimum gratissimum*.

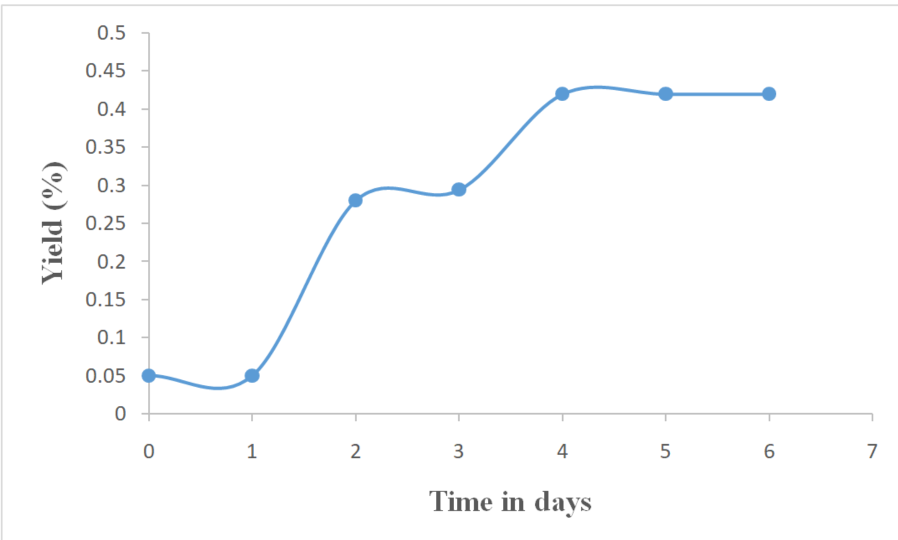


Figure 1. Curve of yield versus drying time.

With drying, a strong increase in the yield of essential oil is obtained to reach 0.294 ± 0.063% on day 3 and a threshold of 0.42 ± 0.08% from day 4 to day 6. Compared to the work of those of [22] carried out, concerning the effect of drying on the chemical composition of the essential oil of *Ocimum basilicum* under the same drying conditions, we find a difference. The yield of the essential oil decreases with drying. According to some authors, the harvest period and the place of harvest have an influence on the yield of essential oil [23, 24]. On the other hand, the increase in yield is similar to that of the essential oil of *Tetraclinis articulate* (Vahl). The increase in EO content from day 2 to day 4, according to Bourkiss and collaborators, is proportional to the humidity level. The HE concentration increases with drying time [25]. This similarity could be explained by the fact that the organs were dried in the same way and the distillation technique is the same: hydrodistillation.

3.2. Phytochemical composition of essential oils extracted by GC-MS according to drying

Table 2. Chemical composition of EO of *Ocimum gratissimum* during drying.

				Percentage based on drying time			
N°	**IR**	**M/Z**	**Compounds**	**% J_0**	**% J_2**	**% J_4**	**% J_6**
1	919	136	α-thujene	1.37	3.11	3.87	3.58
2	924	136	α-pinene	1.47	**0.93**	1.22	1.10
3	937	136	Camphene	-	0.12	0.16	0.13
4	966	136	β-pinene	0.58	0.83	0.98	0.95
5	988	136	β-myrcene	1.36	2.29	2.6	2.59
6	998	136	α-phellandrene	0.15	0.23	0.25	0.45
7	1003	136	4-carene	-	0.14	0.16	0.16
8	1010	136	α-terpinene	1.47	2.19	2.2	2.31
9	**1818**	**136**	**para-cymene**	**1.32**	**12.32**	**25.60**	**24.88**
10	1036	136	(E)-β-ocimene	0.14	0.23	0.26	0.25
11	10,46	136	(Z)-β-ocimene	-	0.11	0.11	0.11
12	**1053**	**136**	**γ-terpinene**	**10.18**	**11.41**	**10.09**	**10.41**
13	1061	154	β-terpineol	0.95	1.25	1.36	1.58
14	10,84	136	dehydro-p-cymene	0.97	1.17	1.24	1.20
15	1091	154	linalol	0.27	0.36	0.36	0.39
16	1097	136	3-carene	0.10	0.18	0.15	0.21
17	1110	152	α-thujone	0.12	0.13	012	0.16
18	1158	154	4-thujanol	-	-	-	0.09
19	1158	154	borneol	0.13	0.44	0.23	0.27
20	1160	154	thujyl alcool	0.17	-	-	-
21	1161	152	ZZ-2,6dimethylocta-3,5,7-trien-2-ol	-	-	0.14	-
22	1161	152	β-terpineol	-	-	-	0,19
23	1170	154	4-carvmenthenol	1.35	2.02	0.74	2.02
24	11231	164	2-isopropyl-5-methylanisole	1.60	-	-	-
25	1180	150	2-(4-methyiphenyl)propan-2-ol	-	0.11	0.12	0.12
26	1184	136	terpinolene	-	0.16	0.13	0.18
27	1231	154	orthomethylthymol	-	1.09	0.95	1.03

28	1266	246	(1,1-dimethyldecyl)-benzene	-	-	-	0.08
29	**1292**	**150**	**thymol**	**40.60**	**44.73**	**33.60**	**35.13**
30	1299	150	carvacrol	1.89	-	1.97	2.35
31	1366	204	α-cubebene	0.40	0.12	0.11	0.08
32	1384	204	longifolene	0.14	-	-	-
33	**1408**	**204**	**β-caryophyllene**	**3.29**	**2.54**	**2.80**	**2.83**
34	1429	204	α-bergamotene	-	-	-	0.07
35	1442	204	α-caryophyllene	0.35	0.26	0.27	0.27
36	1457	190	precocenei	-	-	2.75	0.55
37	1475	204	β-selinene	2.58	1.91	2.40	2.17
38	1484	204	α-elemene	0.99	0.57	0.64	0.67
39	1495	180	2-terbutyl-4-hydroxyanisole	0.78	-	-	-
40	1505	204	germacrened	0.48	0.12	0.12	0.14
41	1515	204	γ-cadinene	0.32	-	-	-
42	1571	220	oxyde de caryophyllene	2.16	1.23	0.0	0.85
43	1597	124	ε-cyclogeraniolene	0.13	-	-	-
44	**2735**	**390**	**bis (2-ethylhexyl)phtalate**	**12.12**	**0.69**	**0.39**	**0.42**
			Hydrocarbons monoterpene	29.11	42.42	49.1	48.51
			Monoterpenes oxygeneted	45.48	50.02	40.33	43.21
			Hydrocarbons sesquiterpène	8.55	5.52	6.34	6.23
			Oxygeneted sesquiterpene	2.16	1.23	0.80	0.85
			Others	13.63	0.8	3.4	1.17
			Total %	**99.93**	**99.99**	**99.97**	**99.97**

IR: Retention Index; M/Z: Molecular weight and % J: day percentage

The analyzes of the different chromatograms and the different mass spectra during drying made it possible to identify 32 to 37 compounds in the different essential oil extracts. The total phytochemical compositions vary from 99.93 to 99.97%.

During the drying, the number of compounds increases from day 0 to day 6 (from 32 to 37 compounds).

The essential oil freshly extracted from leafy twigs of *Ocimum gratissimum* (day 0) contains 32 phytocompounds. The main phytoconstituents are as follows: thymol (40.60%), para-cymene (12.32%), bis (2-ethylhexyl)phthalate (12.12%), γ-terpinene (10.18 %) and β-caryophyllene (3.29%). This composition is characterized by a high presence of oxygenated monoterpenes (45.48%), and a low proportion of oxygenated sesquiterpenes (2.16%). We note the presence of hydrocarbon monoterpenes (29.11%), hydrocarbon sesquiterpenes (8.55%), and 13.63% for the other compounds.

We compared our results with those of Kassi collaborators carried out at the University of Cocody. We note a slight difference in terms of the majority phytocompounds and the number of phytocompounds. They have obtained as major compounds thymol and γ-terpinene and the number of compounds (42) through the method of steam distillation [11]. This difference could be explained by the difference in the extraction method used and the study area. We have also compared our results to those carried out in northern Vietnam by those of Dung and collaborators. We note a large difference in the major compounds and the number of compounds obtained (27). They obtained as major compounds Eugenol, Ocimene, Caryophyllene and Germacrene D [13]. This difference could be explained by the fact that the extraction was made on the fresh leaves and stems. And also because of the difference in the area (place) of harvest.

After the third day of drying (day 2), the essential oil obtained contains approximately the same number of compounds as on day 0. The main compounds are: thymol (44.73%), para-cymene (12 .32%), γ-terpinene (11.41%) and β-caryophyllene (2.54%). Bis (2-ethylenyl) phthalate is in the minority on the second day of drying. This could be explained by the effect of drying the chemical composition. This chemical phyto-composition is also dominated by monoterpenes including 50.02% oxygen and 42.42% hydrocarbons. Oxygenated sesquiterpenes (1.23%), hydrocarbon sesquiterpenes (5.52%), and other 0.8% compounds are in low proportions.

These results are slightly different from the proportions of the first day of drying.

After the fifth day of drying (day 4), the essential oil obtained contains 34 phytocompounds. The main phytoconstituents are: thymol (33.60)%, para-cymene (25.60%), γ-terpinene (10.09%), β-caryophyllene (2.80%). This composition is also dominated by monoterpenes; i.e. 49.10% hydrocarbon monoterpenes and 40.33% oxygenated monoterpenes. Oxygenated (0.80%) and hydrocarbon (6.34%) sesquiterenes are in low proportions. We note 3.4% for the other compounds.

After the seventh day of drying (day 6), the essential oil obtained contains 37 phycompounds. It should be noted that the number of compounds increases according to the day of drying. The major phytocompounds are as follows: thymol (35.13%), para-cymene (24.88%), γ-terpinene (10.41%), β-caryophyllene (2.83%). These compounds are characterized by the presence of hydrocarbon (48.51%) and oxygenated (43.21%) monoterpenes, hydrocarbon (6.23%) and oxygenated (0.85%) sesquiterpenes and 1.17% for the other compounds. We compared our results with those of Koffi and collaborators. the EO of the leaves of the species extracted by hydrodistillation after a week of drying consists of monoterpenes (hydrocarbon and oxygenated) and hydrocarbon sesquiterpenes. The major compounds are p-cymene (37.79%) and thymol (24.757%) [8, 9]. This similarity could be explained by the drying time and the extraction technique.

In view of the results, we note an influence of the drying of the chemical composition of the extracted essential oils.

3.3. Effect of drying on major compounds

Figure 2 represents the variations of the major compounds during the drying. With regard to this result, we note during the drying that: The proportion of thymol (A) is high

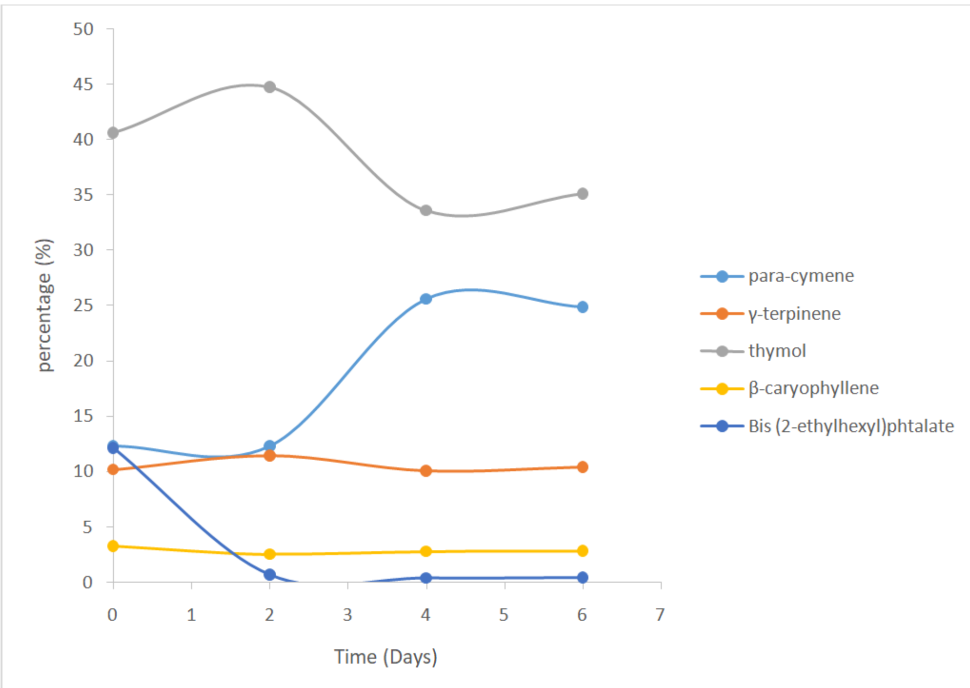
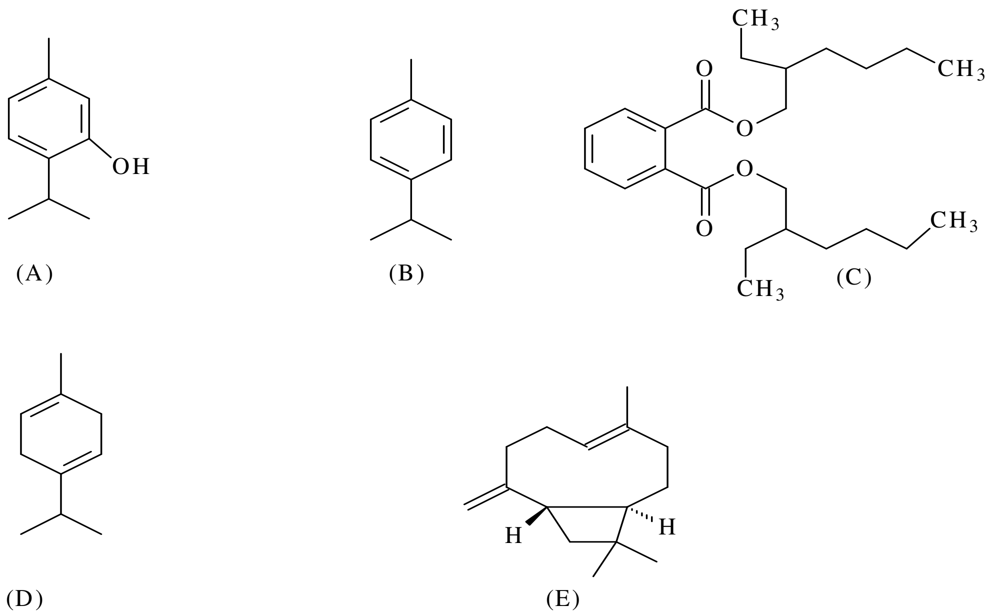


Figure 2. Chemical compositions of the main compounds according to the drying time.

compared to the others during these 7 days of drying, varying from 40.60% on day 0 to 44.73% on day 2 and from 33.60% on day 4 to 35, 15% on day 6.The proportion of para-cymene (B) is constant from day 0 to day 2 (12.32%) and increases to reach 25.60% on day 0 and remains almost constant until day 6 (24.88%). The proportion of Bis (2-ethylhexyl) phthalate (C) is (12.12%) on day 0 and minor on the other days of drying, varying from 0.69% on day 2 to 0.42% on day 6. The proportion of γ-terpinene (D) is almost constant from day 0 to day 6 ranging from 10.18% to 10.40%. The proportion of β-caryophyllene (E) is also almost constant from day 0 to day 6, varying from 3.29% to 2.83%. Figure 3 shows the structures of the majority phytocompounds. All of the variations in the chemical compositions of the majority compounds as a function of drying time are similar to those of [20], having shown that during the drying, the contents of the majority phytocompounds of the essential oil undergo variations.



A : Thymol ; B : para-Cymene ; C : Bis(2-ethylhexyl)phtalate ; D : γ-terpinene ;

E : β-Caryophyllene

Figure 3. Molecular structure of major phytochemical compounds of essential oil of *Ocimum gratissimum*.

4. Conclusion

In this study, we were interested in the valuation of *Ocimum gratissimum*, an aromatic and medicinal plant of the Ivorian flora. This study has shown the importance of drying plant material on the yield and phytochemical composition of the essential oil. It has shown the variation in the yield of the extracted HE as a function of the drying time. Which went from (0.05±0.01)% to (0.42±0.08)%. The number of compounds has increased from 32 to 37. This indicates the variation in the chemical composition of the extracted EO depending on the drying. Hydrocarbon monoterpenes have increased from day 0 to day 4 (29.11% to 49.1%) and decreased to 48.51% by day 6. Oxygenated monoterpenes have increased from day 0 to day 2 (45.48 to 50.02%), decreased from day 2 to day 4 (50.02 to 40.33%) and increased further from day 4 to day 6 (40.33 to 43.21%). Hydrocarbon sesquiterpenes have decreased from day 0 to day 2 (8.55 to 5.52%), increased from day 2 to day 4 (5.52 to 6.34%) and experienced a slight decrease from day 4 to day 6 (6.34 to 6.23%). Oxygenated sesquiterpenes have decreased from day 0 to day 4 (2.16 to 0.80%) and increased slightly to 0.85% on day 6. Other compounds varied. Finally, we also note the variation in the proportions of the majority compounds as a function of drying. In perspective, it would be desirable to see the effect of drying. The biological activities of EO.

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