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Chemical Variability of Essential Oils of *Cyperus rotundus* from Senegal

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Abstract

The composition of 30 samples of essential oil isolated from rhizomes of wild-growing *Cyperus rotundus* harvested in five locations in Senegal was investigated by GC-FID and GC/MS. Essential oils consisted mainly of sesquiterpenes, humulene epoxide 2 (7.0-38.3%), caryophyllene oxide (5.5-28.1%), longiverbenone (3.0-9.4%), cyperotundone (1.2-15.4%), cyperene (0.7-11.1%) and mustakone (1.0-9.1%) being the main components. The compositions of the 30 oils were subjected to k-means partitioning and principal component analysis, which distinguished two groups within the oil samples. The first group encompassed samples collected Ngano and Ziguinchor. The dominant compounds in this group were of humulene epoxide 2, caryophyllene oxide, mustakone and cyperotundone. The second includes specimens collected from Mont-Rollant (M), Ndiayene Pendao (N) and Guede (G), which have a high percentage of caryophyllene oxide and humulene epoxide 2. The second group is distinguished from the first by significantly higher contents of humulene epoxide 2, caryophyllene oxide and, at the same time, lower contents of mustakone and cyperotundone. To our knowledge, the first chemo type has never been described in the literature.

Keywords: *Cyperus rotundus*; Rhizome; Essential Oils; GC-MS; Chemical Variability

Introduction

Cyperus rotundus is a member of Cyperaceae family widely distributed in tropical, subtropical, and temperate regions of the world. This plant grows well in almost any soil type, over a wide range of soil humidity, pH, and altitude. It is annual plant that produces prominent swollen underground tuberous bases that remain dormant after the growing season and under adverse conditions [1-3]. *Cyperus rotundus* is a versatile plant, widely used in traditional medicine around the world and as raw material for perfumes [4-7]. *C. rotundus* rhizome essential oil possessed antioxidant [6,8,9], antibacterial [6,8-14], insecticide [2,15,16], antimutagenic [6,17], anti-inflammatory [7], antiarthritic [7], analgesic [7] and anticonvulsant activities [7].

C. rotundus tubers show great variation in essential oil composition and several chemotypes have been described. Cyperene (19.2-30.9%) and α-cyperone (4.5-25.2%) were the most abundant constituents in the oils of species from Nigeria and Tunisia [17,18]. Chinese, Iranian and Turkish authors have also reported this high content of cyperene. In China, the essential oil was dominated by cyperene (41.03%), β-caryophyllene (5.32%), α-selinene (4.37%) and α-copaene (4.36%)[19]. In Iran the chemotype was cyperene (16.9%), caryophyllene oxide (8.9%), α-longipinan (8.4%) and β-selinene (6.6%) [20]. In Turkey, cyperene (30.5% and 28.0%), α-copaene (10.6% and 12%) and α-ylangene (7.7% and 10.5%) were identified as the main volatile components of rhizomes [21]. Another study conducted in Iran revealed the presence of elemenone (13.59%), α-cyperone (13.14%), and caryophyllene oxide (13.03%) [15]. The Brazilian species contained α-cyperone (22.8%) and cyperotundone (12.1%) as the main in the essential oil compounds [22]. α-Cyperone (21.1%) has also been reported as the main compound of essential oil from Saudi Arabia along with 4-oxo-α-ylangene (12.8%) [2]. Cyperotundone was also present in the essential oil from Iraq which was mainly composed of two compounds namely cyperene (37.9%) and cyperotundone (11.2%) [23]. The rhizome oils of *C. rotundus* from India consisted mainly of α-copaene (11.4-12.1%), cyperene (8.04-11.07%), valerenal (8.07-9.08%) and caryophyllene oxide (7.08-9.07%) [24]. Sonwa and Koenig (2001) studied the essential oil of *C. rotundus* from Germany and found that it was dominated by cyprotene, α-copaene, cyperene, α-selinene and rotundene [25]. More recently, Cisse et al (2021) reported this humulene epoxide 2 (26.1%), caryophyllene oxide (19.2%), longiverbenone (11.3%) chemotype in the essential oil of *C. rotundus* collected in Senegal [26].

The purpose of this study was to determine the chemical composition of *C. rotundus* oils collected in different areas in Senegal and to compare this with those of essential oils obtained from other geographical region.

Material and Methods

Plant material

Thirty *C. rotundus* rhizome samples were collected from five localities in Senegal given in Figure 1: Mont-Rolland (14°55.5988'N, 16°58.59879'W; six samples: M1-M6), Ndiayene Pendao (16°30.24223'N, 15°3.17579'W; seven samples: N1-N7), Guede (16°32.54537'N, 14°45.15816'W; six samples: G1-G6), Ngano (15°16.5628'N, 13°2'.52007'W; five samples: NG1-NG5) and Ziguinchor (12°34'336'N, 16°17'35879'W; six samples: Z1-Z6). Each rhizome sample is taken from an area of approximately 200 m^2. The sampling areas are at least 500 meters apart. The plant material was identified by the technicians from the Botany department of the Fundamental Institute of Black Africa (IFAN) at Cheikh Anta Diop University in Dakar.

Extraction of Essential Oils

Plant samples were air dried for a period of four weeks at room temperature. The plant material was powdered with an average particle size of 0.2 mm using a blade grinder (Polymix PX-MFC 90D, KINEMATICA AG, Luzern, Switzerland). The samples were hydrodistilled (6 h) using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [27].

Figure 1: Sampling of *C. rotundus* rhizomes

Chemical Compositions

The chromatographic analyses were carried out using a Perkin-Elmer Autosystem XL GC apparatus (Walthon, MA, USA) equipped with dual flame ionization detection (FID) system and fused-silica capillary columns, namely, Rtx-1 (polydimethylsiloxane) and Rtx-wax (poly-ethyleneglycol) (60 m × 0.22 mm i.d; film thickness 0.25 µm). The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C for 35 min: hydrogen was employed as carrier gas (1mL/min). The injector and detector temperatures were maintained at 280°C, and samples were injected (0.2 µL of pure oil) in split mode (1:50). The retention indices (RI) of the compounds were determined relative to the retention times of a series of n-alkanes (C5–C30) by linear interpolation using the equation of Van den Dool and Kratz (1963) with the aid of the Perkin-Elmer software (Total Chrom navigator). The relative percentages of the oil constituents were calculated from the GC peak areas, without applying of correction factors.

The samples were also analysed with a Perkin-Elmer Turbo mass detector (quadrupole) coupled to a Perkin-ElmerAutosystem XL, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. The oven temperature was programmed from 60 to 230°C at 2°C/min and then held isothermally at 230°C (35 min): hydrogen was used as carrier gas (1 mL/min). The following chromatographic conditions were employed: injection volume, 0.2 µL of pure oil; injector temperature, 280°C; split, 1:80; ion source temperature, 150°C; ionization energy, 70 eV; MS (EI) acquired over the mass range, 35–350 Da; scan rate, 1 s. Identification of the components was based on: (a) comparison of their GC retention indices (RI) on non-polar and polar columns, determined from the retention times of a series of n-alkanes with linear interpolation, with those of authentic compounds or literature data; (b) on computer matching with commercial mass spectral libraries [28-30] and comparison of spectra with those of our personal library; and (c) comparison of RI and MS spectral data of authentic compounds or literature data.

Statistical Analysis

Data analyses were performed using PCA and CA. Both methods aim at reducing the multivariate space in which objects (oil samples) are distributed, but are complementary in their ability to present results. Indeed, PCA provides the data for plots in which both objects (oil samples) and variables (oil components) are represented, while cluster analysis (CA) informs a classification tree in which objects (sample locations) are gathered. The PCA was carried

out using the function PCA of the statistical XLSTAT software (Addinsoft, Paris). The discriminate variables (volatile components) have been selected using function of the statistical software. A dendrogram was produced by CA using Ward's method of hierarchical clustering, based on Euclidean distances between pairs of oil samples.

Results and Discussion

Yields of essential oils given in Table 1 were calculated on the basis of the mass of dry plant matter. They are between 0.6 and 2.6% (Mean ± SD: 1.19 ± 0.53%). These results are consistent with literature reports indicating yields of 0.20–2.60% [2,6,8-11,14,16,18-20,23,31,32].

Table 1: Chemical variability of the essential oils from *C. rotundus* rhizomes according to sample locations in Senegal

N[a]	Compounds	*l*RI[b]	RIa[c]	RIp[d]	Group I						Group II		
					Subgroup Ia			**Subgroup Ib**					
					M ± SD	**Min**	**Max**	**M ± SD**	**Min**	**Max**	**M ± SD**	**Min**	**Max**
1	*α*-Pinene	931	931	1015	0.2 ± 0.1	0.1	0.2	0.2 ± 0.1	0.1	0.3	0.6 ± 0.3	0.4	1.5
2	Camphene	950	948	1059	0.2 ± 0.1	0.1	0.2	0.1 ± 0.0	0.0	0.1	0.1 ± 0.0	0.0	0.1
3	*β*-Pinene	978	972	1108	0.2 ± 0.1	0.1	0.2	0.1 ± 0.0	0.1	0.1	0.7 ± 0.3	0.4	1.4
4	*p*-Cymene	1015	1013	1264	0.1 ± 0.0	0.0	0.1	0.0 ± 0.0	0.0	0.0	0.1 ± 0.1	0.0	0.5
5	Limonene	1025	1022	1200	0.1 ± 0.0	0.1	0.2	0.1 ± 0.0	0.0	0.1	0.2 ± 0.1	0.1	0.4
6	α-Campholenal	1105	1105	1481	0.1 ± 0.0	0.1	0.1	0.1 ± 0.0	0.1	0.1	0.2 ± 0.1	0.1	0.3
7	Nopinone	1116	1108	1578	0.6 ± 0.1	0.5	0.7	0.2 ± 0.0	0.2	0.2	0.6 ± 0.6	0.1	1.7
8	Cis-verbenol	1132	1131	1655	0.4 ± 0.1	0.3	0.5	0.1 ± 0.0	0.1	0.1	0.5 ± 0.3	0.1	0.9
9	Pinocarvone	1137	1139	1558	0.2 ± 0.1	0.1	0.3	0.1 ± 0.0	0.1	0.2	0.6 ± 0.4	0.2	1.8
10	Myrtenal	1172	1169	1628	0.4 ± 0.2	0.3	0.7	0.3 ± 0.1	0.2	0.4	0.8 ± 0.2	0.6	1.6
11	Trans-Dihydrocarvone	1177	1181	1626	0.4 ± 0.2	0.2	0.7	0.2 ± 0.1	0.1	0.3	0.4 ± 0.3	0.1	1.2
12	Verbenone	1183	1185	1707	0.6 ± 0.1	0.5	0.6	0.1 ± 0.0	0.1	0.2	0.6 ± 0.4	0.0	1.4
13	Cuminaldehyde	1213	1212	1782	0.1 ± 0.0	0.1	0.1	0.1 ± 0.1	0.0	0.1	0.1 ± 0.2	0.0	0.7
14	Transcarveol	1200	1202	1824	0.2 ± 0.1	0.1	0.3	0.3 ± 0.1	0.2	0.4	0.1 ± 0.1	0.0	0.2
15	Carvone	1214	1216	1739	0.1 ± 0.0	tr	0.1	0.1 ± 0.1	0.0	0.1	0.2 ± 0.1	0.1	0.5
16	*α*-Ylangene	1374	1371	1476	1.3 ± 0.3	1.0	1.7	0.8 ± 0.1	0.7	1.0	0.5 ± 0.1	0.4	0.6
17	*α*-Copaene	1379	1375	1488	1.4 ± 0.3	1.1	1.6	1.5 ± 0.4	1.0	2.1	1.9 ± 0.5	1.1	2.9
18	β-Elemene	1389	1386	1589	0.5 ± 0.1	0.4	0.7	0.4 ± 0.1	0.3	0.4	0.3 ± 0.1	0.1	0.4
19	Sativene	1395	1393	1529	0.1 ± 0.0	0.1	0.2	0.6 ± 0.8	0.1	1.8	0.2 ± 0.2	0.0	0.5
20	**Cyperene**	**1402**	**1400**	**1531**	**8.5 ± 1.8**	**6.5**	**11.1**	5.6 ± 0.5	5.1	6.6	1.5 ± 0.6	0.7	2.9
21	β-Caryophyllene	1421	1417	1583	0.5 ± 0.1	0.3	0.6	0.2 ± 0.1	0.1	0.2	0.3 ± 0.1	0.2	0.4
22	Humulene	1455	1450	1660	0.7 ± 0.1	0.6	0.9	0.3 ± 0.1	0.2	0.4	0.9 ± 0.5	0.3	1.7
23	Rotundene	1461	1456	1629	2.0 ± 0.2	1.7	2.3	1.9 ± 0.3	1.5	2.3	0.6 ± 0.4	0.2	1.3
24	Alloaromadendrene	1462	1462	1638	0.5 ± 0.1	0.4	0.6	0.4 ± 0.1	0.3	0.4	0.2 ± 0.1	0.1	0.4
25	*γ*-Muurolene	1474	1470	1681	1.4 ± 0.4	1.0	2.0	0.4 ± 0.1	0.3	0.6	0.8 ± 0.3	0.1	1.2
26	Germacrene D	1479	1476	1704	0.1 ± 0.0	0.0	0.1	0.1 ± 0.0	0.1	0.1	0.0 ± 0.0	0.0	0.0
27	*β*-guaiene	1482	1484	1719	0.3 ± 0.0	0.3	0.4	0.2 ± 0.1	0.1	0.3	0.1 ± 0.1	0.0	0.6
28	Germacrene A	1484	1485	1695	0.3 ± 0.1	0.2	0.4	0.6 ± 0.1	0.5	0.6	0.2 ± 0.2	0.0	0.7

29	α-Bulnesene	1503	1494	1711	0.9 ± 0.1	0.8	1.1	0.5 ± 0.1	0.4	0.6	0.5 ± 0.1	0.4	0.7
30	β-Bisabolene	1503	1501	1718	0.1 ± 0.0	0.1	0.2	0.2 ± 0.1	0.1	0.2	-	-	-
31	δ-Cadinene	1520	1514	1746	1.1 ± 0.3	0.6	1.3	1.4 ± 0.1	1.2	1.6	0.6 ± 0.2	0.3	1.
32	α-Calocorene	1527	1528	1895	0.6 ± 0.2	0.5	0.9	0.5 ± 0.1	0.4	0.6	0.3 ± 0.1	0.1	0.4
33	β-Calocorene	1545	1548	1902	-	-	-	0.3 ± 0.2	0	0.5	0.1 ± 0.1	0.0	0.3
34	Nootkatene	1512	1509	1812	0.7 ± 0.2	0.4	1.0	0.5 ± 0.1	0.3	0.6	0.5 ± 0.1	0.2	0.6
35	Salvidienol	1541	1540	1980	1.3 ± 0.3	1.1	1.8	1.1 ± 0.1	1.0	1.3	1.6 ± 0.3	1.1	2.2
36	Epiglobulol	1552	1562	2037	0.5 ± 0.1	0.4	0.6	1.0 ± 0.3	0.7	1.4	0.3 ± 0.2	0.0	0.6
37	Spathulenol	1572	1560	2119	0.2 ± 0.1	0.1	0.2	1.9 ± 0.7	1.2	3.0	1.0 ± 0.1	0.7	1.2
38	**Caryophyllene oxide**	**1570**	**1573**	**1959**	6.3 ± 0.9	5.5	7.8	6.8 ± 0.9	5.7	7.8	**23.2 ± 2.9**	**18.0**	**28.1**
39	β-Copaen-4-α-ol	1572	1583	2141	1.4 ± 0.2	1.1	1.7	1.2 ± 0.1	1.1	1.3	0.8 ±0 .3	0.2	1.2
40	**Humulene epoxide 2**	**1602**	**1598**	**2044**	**22.6 ± 0.6**	**21.9**	**23.5**	7.8 ± 0.9	6.5	8.6	**32.4 ± 3.3**	**26.4**	**38.3**
41	Caryophylla- 4 (14), 8 (15)-dien-5α-ol	1620	1628	2285	1.3 ± 0.2	1.1	1.5	1.9 ± 0.3	1.5	2.3	0.7 ± 0.4	0.3	1.6
42	**Longiverbenone**	**1644**	**1652**	**2230**	5.0 ± 0.3	4.5	5.3	8.4 ± 1.1	6.6	9.4	5.2 ± 1.9	3.0	10.8
43	Amorpha-4,7(11)-diene-3-one	1667	1664	2245	3.2 ± 0.4	2.8	3.8	3.2 ± 0.4	2.8	3.8	2.4 ± 0.9	1.1	3.9
44	**Mustakone**	**1669**	**1667**	**2270**	3.2 ± 0.6	2.3	4.0	**8.6 ± 0.3**	**8.2**	**9.1**	2.2 ± 0.5	1.0	2.9
45	**Cyperotundone**	**1671**	**1673**	**2278**	**11.5 ± 1.5**	**10.2**	**14.0**	**12.5 ± 1.5**	**10.7**	**15.1**	1.4 ± 0.1	1.2	1.6
46	Ylangenal	1675	1677	2300	1.5 ± 0.3	0.9	1.8	3.7 ± 0.3	3.3	4.3	0.5 ± 0.1	0.4	0.6
47	Cyperenal	1765	1735		1.6 ± 0.1	1.5	1.7	2.6 ± 0.2	2.3	2.8	1.1 ± 0.2	0.9	1.5
48	Cyclocolorenone	1751	1765	2348	1.4 ± 0.2	1.1	1.7	1.9 ± 0.1	1.8	2.1	0.7 ± 0.2	0.5	1.4
49	α-Cyperone	1758	1778	2358	0.3 ± 0.1	0.3	0.4	1.3 ± 0.3	1.1	1.8	0.4 ± 0.1	0.3	0.5
Hydrocarbon monoterpenes					0.7 ± 0.1	0.6	0.8	0.5 ± 0.1	0.4	0.6	1.7 ± 0.6	1.0	3.3
Oxygenated monoterpenes					3.1 ± 0.4	2.6	3.7	1.6 ± 0.2	1.4	1.8	4.1 ± 1.5	1.8	7.4
Hydrocarbon sesquiterpenes					21.1 ± 3.2	17.1	25.2	16.2 ± 1.0	15.0	17.3	9.6 ± 1.7	7.2	12.6
Oxygenated sesquiterpenes					61.1 ± 2.9	58.9	66.0	63.9 ± 2.4	61.5	68.0	73.8 ± 4.7	67.4	81.5
Total identified (%)					85.9 ± 1.8	84.0	88.5	82.1 ± 1.7	80.6	85.2	89.1 ± 3.4	81.9	94.0
Yields (w/w vs dry material)					1.1 ± 0.1	1.0	1.2	0.9 ± 0.1	0.8	1.0	1.2 ± 0.5	0.6	2.6
[a] Order of elution is given on non polar column (Rtx-1).													
[b] Retention indices of literature on the non polar column (lRIa) [29].													
[c] Retention indices on the nonpolar Rtx-1 column (RIa).													
[d] Retention indices on the polar Rtx-Wax column (RIp).													

The analysis of the rhizome essential oils by GC/-FID and GC/MS allowed the identification of 49 compounds accounting for 81.0 to 94.0% of the total compositions (Table 1). These essential oils mainly consisted of high amounts of sesquiterpenes. However, the main compounds varied considerably from one sample to another: humulene epoxide 2 (7.0-38.3%), caryophyllene oxide (5.5-28.1%), longiverbenone (3.0-9.4%), cyperotundone (1.2-15.4%), cyperene (0.7-11.1%) and mustakone (1.0-9.1%) (Figure 2). Thus, statistical analyses were performed on the 30 oil compositions to highlight the chemical variability. Figure 3 was obtained from the correlation matrix calculated with the standardized matrix. As shown in Figure 3, the principal factorial plane (constructed with axes 1 and 2) summarizes 78.57% of the entire variability of essential oils. The distribution of oil samples from C. rotundus oils is reported in Figure 3a. The distribution of variables (volatile components) is shown in Figure 3b (11 variables). The graph drawn using

PCA suggested the existence of two main groups of essential oils (Figure 3a). Statistical analysis using CA was also used to show the correlation between the chemotypes and the geographical distribution of samples. The dendrogram obtained by cluster analysis (CA) reinforced the clustering observed using PCA by grouping the 30 oil samples from C. articulatus into the same two main clusters (Figure 4).

The first group included the samples collected in Ngano (NG) and Ziguinchor (Z). The dominant compounds in this group were of humulene epoxide 2, caryophyllene oxide, mustakone and cyperotundone. This group was divided into two subgroups. The first subgroup, including Ngano samples, showed a high content of humulene epoxide 2 (21.9-23.5%, mean: 22.6%, SD: 0.6%) and cyperotundone (10.2-14.0%, mean: 11.5%, SD: 1.5%). Instead, the high content of mustakone and cyperotundone was a feature of the second subgroup, which included Ngano samples. To our knowledge, these chemotypes have never been described in the literature. However, the presence of mustakone and cyperotundone at significant levels has been reported in a study conducted in Tunisia [8,17]. Cyperotundone has also been described in essential oils of *C. rotundus* in Asia [32], Iran [20], Marocco [12] and Brazil [22].

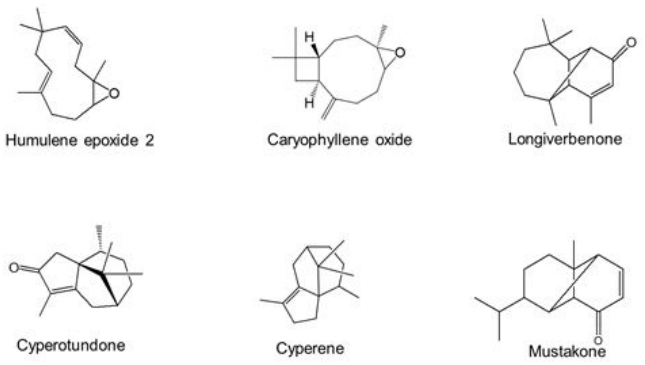

Figure 2: Chemical structures of major compounds

The second group includes specimens collected from Mont-Rollant (M), Ndiayene Pendao (N) and Guede (G), which have a high percentage of caryophyllene oxide (18.0-28.1%, mean: 23.2%, SD: 2.9%), humulene epoxide 2 (26.4-38.3, mean: 32.4%, SD: 3.3%). Similarly, high contents of humulene epoxide 2 and caryophyllene oxide have been reported for *C. rotundus* oil from Louga region in Senegal. However, these compounds were at low levels or absent in essential oils of *C. rotundus* from Iran [20], India [24] and Brazil [24].

In summary, the results obtained from the analysis of the thirty samples have led to the establishment of three chemotypes to *C. rotundus* essential oils. These results are based on single samples to each collection site and do not account for within site variation. However, the chemical composition of the analyzed essential oils showed qualitative and quantitative variation by the influence of the localization.

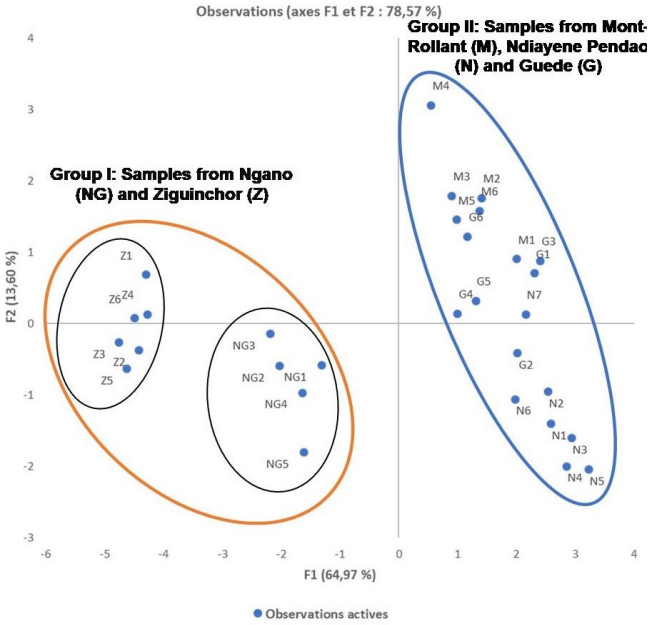

Figure 3A: Distribution of samples

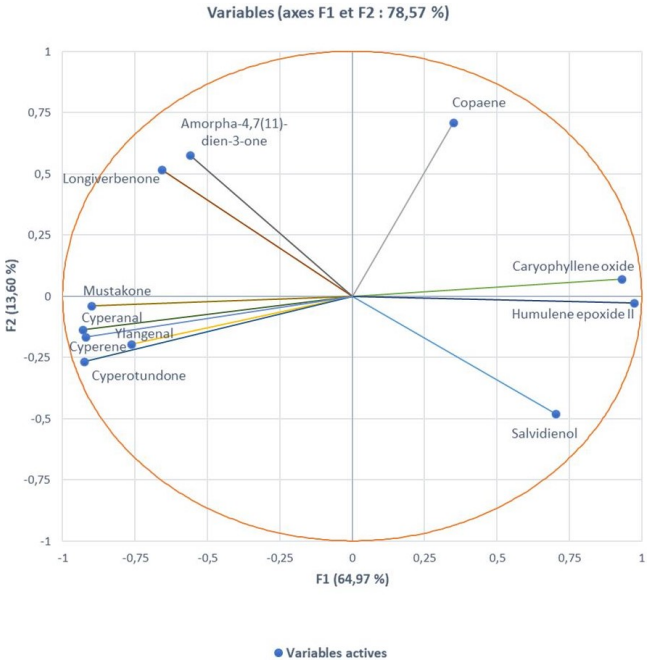

Figure 3B: Distribution of variables

Figure 3: Principal component analysis of chemical composition of the *C. rotundus* rhizome oils from Senegal

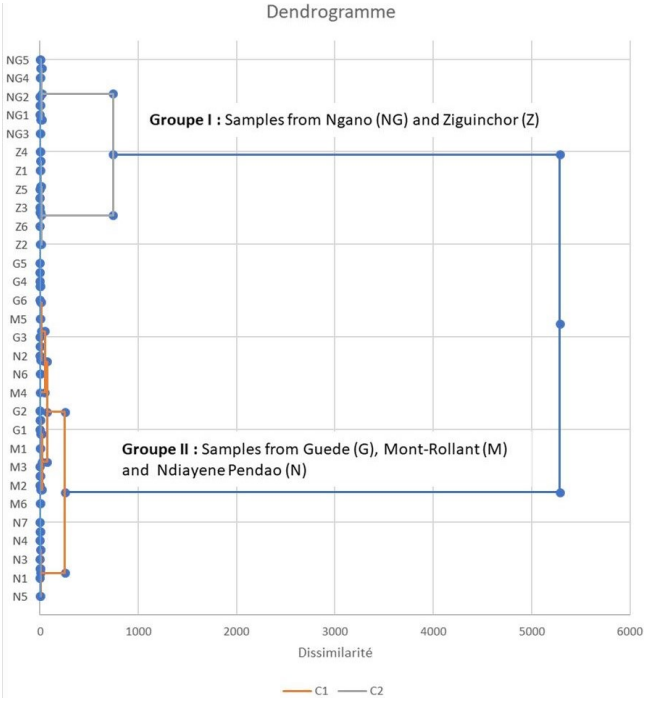

Figure 4: CA of chemical composition of the essential oils from *C. rotundus* rhizomes

Conclusion

The present investigation demonstrated the important of chemical variability of the essential oils isolated from the rhizomes of *C. rotundus* populations growing wild in Senegal. Principal component analysis (PCA) and cluster analysis (CA) (dendrogram) applied on the matrix linking essential oil compositions and sample locations allowed the distinction of two groups within the oil samples. The dominant compounds in the first group were humulene epoxide 2, caryophyllene oxide, mustakone and cyperotundone and those in the second group were caryophyllene oxide and humulene epoxide 2. To our knowledge, we have reported the first chemotype for the first time. In perspective, we will evaluate their biological activities and also produce cosmetic formulations based on these essential oils.

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