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Chemical constituents of essential oils from flowers and stems of an Ivorian species of the genus Crassocephalum

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Abstract
Essential oils from fresh flowers and stems of *Crassocephalum sp* (Asteraceae) growing wild in Côte d'Ivoire were extracted by hydrodistillation using a Clevenger-type apparatus. Essential oils were analyzed by Gas Chromatography (GC) and then by GC coupled with a Mass Spectrometry (GC-MS). The analyzes of the oils allowed the identification and quantification of 39 and 83 constituents respectively in the oils of the flowers and the stems. The characterization of the oils revealed the predominance of the hydrocarbon monoterpene myrcene with 87.25% in the flowers, 35.55% in the stems. Myrcene alone accounts for almost all of the flower oil. The chemotype of the stem oils is different from that of the flowers with the presence of terpinolene (31.99%) and (*E*)-dec-2-enal (8.01%) which are other major compounds in the oils of the stems of *Crassocephalum sp.*

Keywords: *Crassocephalum*, chemotype, essential oils, phytochemistry, myrcene, terpinolene

Introduction
The genus Crassocephalum to which the species *Crassocephalum sp* belongs, is part of the very large and widespread family Asteraceae in the tribe Senicioneae. The genus Crassocephalum has been reported to include nearly 24 species (Wagner *et al.*, 1999) [10] some of which have medicinal properties. Several species of Crassocephalum are used in traditional medicine for their therapeutic virtue. *Crassocephalum bauchiense* Huch is widely used in Cameroonian traditional medicine. The leaf extract has been used to treat several illnesses, including epilepsy, arthritis, intestinal pain and colic (Arbonnier, 2009) [2]. A decoction of the leaves of this herb has been reported to be useful in relieving bronchitis and accompanying fever. According to traditional Cameroonian healers, the plant is also effective in cases of brain deficit, behavioral disorders in mentally retarded children, inflammatory disorders and neuropathic pain (Taïwe *et al.*, 2012) [9]. Among the species of the genus Crassocephalum, several studies on the chemical composition of essential oils have been carried out for the species *Crassocephalum crepidiodes*. In Vietnam, the main components of essential oils extracted from *Crassocephalum crepidioides* flowers are myrcene (43.3%), *β*-phellandrene (10.7%) and cryptone (8.1%) (Hung *et al.*, 2019) [3]. The chemical composition of the essential oils of the stems differs slightly with those of the flowers. Stem oils have as predominant compounds myrcene (26.1%), α-pinene (10.7%), α-humulene (5.9%) and (*E*)-*β*-farnesene (5.2%) (Hung *et al.*, 2019) [3]. On the other hand, the essential oils of the flowers and aerial parts (leaves + stems) of Crassocephalum crepidiodes. harvested in western India (Joshi, 2011) [5] had chemical compositions close to those of Vietnam. The essential oils extracted from the flowers of India contain 46.1% myrcene and 31.0% *β*-phellandrene and the essential oils from the aerial parts contain 45.3% of myrcene and 20.2% of *β*-phellandrene (Joshi, 2011) [5]. Cryptone, which was relatively abundant in essential oils from Vietnam, was only observed in small proportions (0.1%) in oils from India. In Africa, essential oils from the leaves of *C. crepidioides* from Cameroon had *α*-phellandrene, *p*-cymene, myrcene, limonene and (*E*)-*β*-ocimene as major constituents (Zollo *et al.*, 2000) [11]. In another report from Nigeria, the important constituents of oils from the leaves of the same plant were α-caryophyllene, *β*-cubebene and α-farnesene; while thymol, α-caryophyllene and 4-cyclohexybutyramide were the main constituents of stem oil (Owokotomo *et al.*, 2012) [7]. It should be noted the absence of studies on the essential oils extracted from the flowers and stems of a species of the genus

Crassocephalum in Africa. It is important to note that no chemical study has been carried out on the volatile secondary metabolites (essential oils) of the flowers and stems of a species of the genus Crassocephalum in Côte d'Ivoire. Our research therefore presents for the first time, in Côte d'Ivoire, the chemical composition of essential oils extracted from the flowers and stems of a plant of the genus Crassocephalum, the species *Crassocephalum sp* using a combination of three GC and GC/MS techniques.

Materials and Methods

Plant Materials

Flowers and stems of *Crassocephalum sp* were collected at 8 am in the fallow lands of the village of Gabia in the Oumé sub-prefecture, Gôh region (Centre-West of Côte d'Ivoire). Plant materials were identified in the National Floristic Center of University of Felix Houphouët Boigny, Cocody-Abidjan, department of Botany, Côte d'Ivoire.

Isolation of Essential Oil

Extraction of essential oils was carried out by steam distillation using a Clevenger-type apparatus. Fresh Flowers or stems (1000 g) were placed on a metal grid under which is distilled water (3000 mL) boiling for 4 hours. Oils are driven by steam. After condensation and liquefaction, the oil overcomes the water in the test tube. The obtained oil was dried over anhydrous sodium sulphate and after filtration, stored in a sealed sample tube at 0 °C until GC and GC-MS analysis.

Chemical Analyses of the essential oil

The essential oil was investigated first by Gas Chromatography (GC) and then by GC coupled with a Mass Spectrometry (GC-MS). Concerning GC analysis, the gas phase chromatography was carried out using a Delsi DI 200 instrument equipped with a flame ionization detector and a DB5 column (25 m x 0.25 mm, df: 0.25 µm) with a split flow rate of 60 mL/min. Nitrogen was used as carrier gas; temperature programming was 5min at 50 °C and 30 °C/min up to 220 °C, injector and detector temperatures were respectively set to 220 °C and 250 °C. GC-MS analysis were performed using a Hewlett-Packard gas Chromatograph Model 6890 coupled to a Hewlett-Packard MS Model 6890 equipped with an HP5 column (30 m x 0.25 mm, df: 0.25 µm). Initial oven temperature was maintained at 50 °C for 5 min and then programmed at 50 °C/min to 300 °C (held 50 min). The carrier gas was helium (1.0 mL/min); a split injection with a split ratio 1:10 was chosen. Injector and detector temperatures were respectively set to 250 °C and 320 °C. The electron multiplier was set at 2200 V with an applied electron ionization voltage of 70 eV, with the ion source temperature at 230 °C. Mass spectral data were acquired in the scan mode in the m/z range of 33-450. Identification of compounds was carried out by calculating Retention Indices (RI) or Kovàts Indices (KI) and comparing mass spectra with those in data banks, i.e. Adams (Adams, 1995) [1] or Mc Lafferty and Stauffer (Mc Lafferty *et al*., 1989) [6]. For quantification purpose, relative area percentages obtained by field ionization detection (FID) were used.

Results and Discussions

The essential oils obtained by hydrodistillation of the flowers and stems of Crassocephalum sp from Côte d'Ivoire are yellow in color. The identification and quantification by GC and GC-MS techniques of the essential oils of the flowers of *Crassocephalum sp*, gave a total of 39 major components representing 99.72 Essential oils of the stems have been identified 83 constituents representing 99.19% (Table 1). Monoterpenes (98.43% in the leaves, 74.58% in the stems) dominate by their presence the chemical composition of the essential oils extracted from the flowers and stems of *Crassocephalum sp* from Côte d'Ivoire. The hydrocarbon monoterpene, myrcene (87.25%), is the predominant compound which alone accounts for almost all of the flower oil. Limonene (4.27%) and β-pinene (3.80%), which are also hydrocarbon monoterpenes, are relatively important in proportion compared to the rest of the constituents. Myrcene, limonene and β-pinene together represent 95.32% of the total oil composition of the flowers. Essential oils extracted from *Crassocephalum sp* flowers from Côte d'Ivoire indicate a monoterpene hydrocarbon chemotype. Another chemotype was obtained for the essential oils extracted from the stems. The main components are myrcene (35.55%), terpinolene (31.99%), (*E*)-dec-2-enal (8.01%), *β*-bourbonene (3.28%) and limonene (2.23%). Terpinolene, in small quantity in the flowers 0.83%, is found with a very high content in the stems. The chemical compositions of essential oils from flowers and stems of the species *Crassocephalum crepidioides* collected in Vietnam (Hung *et al.*, 2019) [3], India (Joshi, 2011) [5], Cameroon (Zollo *et al.*, 2000) [11] and in Nigeria (Owokotomo *et al.*, 2012) [7] are different from those obtained in our study on flowers and stems of *Crassocephalum sp* from Côte d'Ivoire. In Vietnam (Hung *et al.*, 2019) [3], oils extracted from flowers and stems also contain myrcene as the predominant compound but with different proportions. Myrcene content in oils obtained from flowers and stems of *Crassocephalum sp* from Côte d'Ivoire are higher than those from flowers and stems from Vietnam. In India, the proportion of myrcene in the aerial part (leaves + stems) is greater than that of the stems of *Crassocephalum sp* in Côte d'Ivoire. The other major compounds reported in Vietnam and India are different from those of the plant in our study. In addition, the compounds in significant amounts in *Crassocephalum crepidioides* oils from Vietnam and India, *β*-phelladrene and cryptone were not identified in our study. The results obtained in our work on the flowers and stems of the species *Crassocephalum sp* from Côte d'Ivoire show different chemotypes to those reported for the oils extracted from the leaves and stems of *Crassocephalum crepidoides* from Nigeria, a country of the West Africa. The thymol (43.93%), *α*-caryophyllene (15.16%) and 4-cyclohexybutyramide (20.94%) chemotype reported for essential oils from the stems of *Crassocephalum crepidoides* from Nigeria (Owokotomo *et al.*, 2012) [7] presents majority compounds which are in the minority or absent in the oils from the stems of the species studied, of the genus Crassocephalum. In Cameroon, the reported α-phellandrene, *p*-cymene, myrcene, limonene and (*E*)-*β*-ocimene chemotype for seven species of Crassocephalum (Zollo *et al.*, 2000) [11] is different from the myrcene chemotype obtained in the essential oils of the flowers and of the myrcene, terpinolene, (*E*)-dec-2-enal chemotype identified in the essential oils of the stems of the species Crassocephalum sp studied. Biological activity studies on several monoterpenes have demonstrated that myrcene has antibacterial properties (Rasoul *et al.*, 2012) [8]. In addition, Inoue *et al.*, (2004) [4] also showed that myrcene contributes to the antibacterial activity of essential oils of *Melaleuca alternifolia* (Inoue *et al.*, 2004) [4]. Myrcene, which is the major constituent of the essential oils of the flowers and stems of the *Crassocephalum sp* plant from Côte d'Ivoire, could transmit its antibacterial properties to the oils of the flowers and stems of the species Crassocephalum sp.

Table 1: Chemical constituents of essential oil of leaves and stems of *Crassocephalum sp*

				Flower	Stem	
No	**KI**	**RI**	**Compounds**	**Contents%**		**Formula**
1	885	886	styrene	0.02	0.18	C_8H_8
2	909	900	2-heptanol	nd	0.10	$C_7H_{16}O$
3	922	920	tricyclene	0.02	0.05	$C_{10}H_{16}$
4	926	929	α-Pinene	0.36	0.05	$C_{10}H_{16}$
5	945	944	camphene	0.01	nd	$C_{10}H_{16}$
6	966	971	sabinene	0.39	0.10	$C_{10}H_{16}$
7	978	976	β-Pinene	3.80	0.79	$C_{10}H_{16}$
8	983	982	myrcene	87.25	35.55	$C_{10}H_{16}$
9	987	988	Yomogi alcohol	0.02	nd	$C_{10}H_{18}O$
10	996	996	2,2,4,6,6-pentamethylheptane	nd	0.20	$C_{12}H_{26}$
11	1007	1005	*α*-phellandrene	0.04	0.01	$C_{10}H_{16}$
12	1018	1011	α-Terpinene	0.03	0.12	$C_{10}H_{16}$
13	1025	1014	paracymene	0.04	1.31	$C_{10}H_{16}$
14	1022	1028	limonene	4.27	2.23	$C_{10}H_{16}$
15	1035	1027	(Z)-β-ocimene	0.02	0.03	$C_{10}H_{16}$
16	1047	1038	(E)-β-ocimene	1.01	0.48	$C_{10}H_{16}$
17	1060	1050	γ-Terpinene	0.07	1.15	$C_{10}H_{16}$
18	1074	1073	p-cymenene	nd	0.22	$C_{10}H_{16}$
19	1081	1083	linalool	0.01	0.05	$C_{10}H_{18}O$
20	1083	1085	trans-linalool oxide	0.01	0.08	$C_{10}H_{18}O_2$
21	1089	1088	terpinolene	0.83	31.99	$C_{10}H_{16}$
22	1095	1098	α-Pinene oxide	0.04	0.04	$C_{10}H_{16}O$
23	1113	1105	(*E*)-4,8-dimethyl-nona-1,3,7-triene	nd	0.04	$C_{11}H_{18}$
24	1163	1162	Terpinen-4-ol	0.04	0.22	$C_{10}H_{18}O$
25	1167	1169	1,4-Dimethoxybenzene	nd	0.06	$C_6H_4(OCH_3)_2$
26	1180	1173	α-Terpineol	0.01	0.03	$C_{10}H_{18}O$
27	1186	1185	decanal	0.16	0.16	$C_{10}H_{20}O$
28	1213	1215	Cumin aldehyde	nd	0.13	$C10H_{12}O$
29	1237	1234	chavicol	nd	0.02	$C_9H_{10}O$
30	1272	1269	(E)-dec-2-enal	0.44	8.01	$C_{10}H_{18}O$
31	1283	1278	carvacrol	nd	0.29	$C_{10}H_{14}O$
32	1286	1285	bornyl acetate	nd	1.29	$C_{12}H_{20}O_2$
33	1290	1289	Thymol	nd	0.33	$C_{10}H_{14}O$
34	1294	1297	nonyl acetate	nd	0.09	$C_{11}H_{22}O_2$
35	1302	1299	Methyl geranate	nd	0.06	$C_{11}H_{18}O_2$
36	1323	1326	γ-Nonalactone	nd	0.03	$C_9H_{16}O_2$
37	1348	1345	Silphin-1-ene	nd	0.46	$C_{15}H_{24}$
38	1353	1352	*α*-Cubebene	0.03	nd	$C_{15}H_{24}$
39	1363	1362	(E)-β-Damascenone	nd	0.02	$C_{13}H_{18}O$
40	1368	1369	cyclosativene	nd	0.05	$C_{15}H_{24}$
41	1375	1375	*α*-copaene	nd	0.09	$C_{15}H_{24}$
42	1380	1381	Modhephene	0.03	0.74	$C_{15}H_{24}$
43	1383	1383	*β*-cubebene	nd	0.02	$C_{15}H_{24}$
44	1388	1387	*β*-bourbonene	0.13	3.28	$C_{15}H_{24}$
45	1393	1391	*β*-Elemene	nd	0.02	$C_{15}H_{24}$
46	1399	1400	Cyperene	nd	0.40	$C_{15}H_{24}$
47	1405	1406	*α*-Barbatene	0.06	1.89	$C_{15}H_{24}$
48	1419	1417	(*E*)-*β*-caryophyllene	0.04	0.94	$C_{15}H_{24}$
49	1430	1428	β-copaene	nd	0.11	$C_{15}H_{24}$
50	1445	1446	Sesquisabinene	nd	0.03	$C_{15}H_{24}$
51	1449	1450	*α*-humulene	0.02	0.43	$C_{15}H_{24}$
52	1474	1475	trans-cadina-1(6),4-diene	0.09	0.02	$C_{15}H_{24}$
53	1480	1481	germacrene-D	0.02	0.24	$C_{15}H_{24}$
54	1491	1491	(Z)-α-Bisabolene	0.10	0.91	$C_{15}H_{24}$
55	1494	1495	(E),(E)-α-Farnesene	nd	0.32	$C_{15}H_{24}$
56	1506	1507	*trans*-Calamenene	0.01	1.33	$C_{15}H_{22}$
57	1512	1510	7-epi-α-Selinene	nd	0.11	$C_{15}H_{24}$
58	1514	1514	δ-Cadinene	0.01	0.16	$C_{15}H_{24}$
59	1535	1534	*trans*-cadina-1,4-diene	nd	0.16	$C_{15}H_{24}$
60	1542	1542	Selina-3,7(11)-diene	nd	0.09	$C_{15}H_{24}$
61	1548	1547	*β*-Elemol	nd	0.03	$C_{15}H_{26}O$
62	1551	1550	Elemol	nd	0.16	$C_{15}H_{26}O$
63	1563	1563	(*E*)-Nerolidol	nd	0.19	$C_{15}H_{26}O$
64	1568	1569	Germacrene-D-4-ol	nd	0.32	$C_{15}H_{26}O$
65	1577	1577	caryophyllene oxide	nd	0.03	$C_{15}H_{26}O$

66	1583	1582	Spathulenol	nd	0.06	$C_{15}H_{26}O$
67	1594	1593	Viridiflorol	nd	0.10	$C_{15}H_{26}O$
68	1601	1601	α-Humulene oxide	nd	0.02	$C_{15}H_{24}O$
69	1610	1607	humulene-1,2-epoxide	nd	0.04	$C_{15}H_{24}O$
70	1615	1614	1,10-di-epi-cubenol	nd	0.12	$C_{15}H_{26}O$
71	1625	1629	10-*epi*-γ-Eudesmol	nd	0.08	$C_{15}H_{26}O$
72	1630	1631	*β*-Eudesmol	0.02	0.15	$C_{15}H_{26}O$
73	1635	1635	*α*-Cadinol	nd	0.14	$C_{15}H_{26}O$
74	1636	1637	*α*-Eudesmol	nd	0.08	$C_{15}H_{26}O$
75	1651	1653	*β*-Bisabolol	nd	0.04	$C_{15}H_{26}O$
76	1676	1677	andro encecalinol	nd	0.07	$C_{12}H_{12}O_2$
77	1698	1695	β-Sinensal	nd	0.12	C15H22O
78	1715	1715	Mayurone	nd	0.02	$C_{14}H_{22}O$
79	1783	1786	Cis-Cadina-1(2),4-diene	0.05	0.03	$C_{15}H_{24}$
80	1834	1830	6,10,14-Trimethyl pentandecan-2-one	nd	0.06	$C_{18}H_{36}O$
81	1862	1866	1-Hexadecanol	nd	0.08	$C_{16}H_{34}O$
82	1899	1884	nonadecane	nd	0.07	$C_{19}H_{40}$
83	1955	1942	isophytol	0.14	0.38	$C_{20}H_{40}O$
84	1978	1976	Ethyl hexadecanoate	0.05	0.02	$C_{18}H_{36}O_2$
85	2107	2106	(E)-phytol	0.04	0.84	$C_{20}H_{40}O$
			Total	99.72	99.19	

nd: no detected

Conclusion

This research work contributes to the promotion of tropical plant species and little-known plants of the genus Crassocephalum. This study presents for the first time phytochemical results ondf essential oils extracted from the flowers and stems of a species of the genus Crassocephalum harvested in Côte d'Ivoire. Our work shows a myrcene, limonene and *β*-pinene monoterpene chemotype for the oils extracted from the flowers and a myrcene, terpinolene and (*E*)-dec-2-enal chemotype for the oils extracted from the stems. Myrcene, which represents almost all of the volatile matter in the oils of the flowers of *Crassocephalum sp*, is also the main compound of the species Crassocephalum crepidioides with a lower percentage. The high amount of myrcene, an antibacterial, in the oils extracted from the flowers of *Crassocephalum sp* could indicate a strong antibacterial activity of the flower oil.

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