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Chemical composition and antioxidant activity of the essential oil of *Croton dybowskii* Hutch from Congo-Brazzaville

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ABSTRACT

The chemical composition of the essential oil of *Croton dibowskii* Hutch from Congo-Brazzaville was analyzed by GC/FID and GC/SM. The chemical profile remains, for the most part, dominated by the cyperene (15.1%), borneol (12.5%), (6.9)-guaiadiene (8.2%), α-muurolene (7.9%) and 1.8-cineole (6.7%), representing in general 57.4% of sesquiterpene hydrocarbons and 23.8% of oxygenated monoterpne. We note in some respects, with a low content, the presence of monoterpenic hydrocarbons and sesquiterpenic oxygenated compounds of 5.0 and 3.0%, respectively. The antioxidant activity on the DPPH turns out to be very limited, it reaches inhibition percentage of 35% from the concentration of 2000 µg/ml with a CI50 estimated at 3000 µg/ml.

Keywords: Antioxidant activity, chemical composition, *Croton dybowskii*, essential oil.

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INTRODUCTION

Croton dybowskii Hutch. (Euphorbiaceae) is a small tree, which is sometimes sarmentose, distributed along the coastal Lower Guinean (Figure 1) (Lebrun and Stork, 2012; Miabangana et al., 2017). Ecologically, *C. dybowskii* is a plant confined in inshore thickets from 2 to 6 m high, with typical species like *Manilkara obovata* (Sabine & G. Don) J.H. Hemsl, in the form of enclaves in the grassy formations in *Anadelphia hamata* Stapf. The typical tree species of the tree and shrub stratum are: *Baphia vili* Cheek, *Barteria nigritana* Hook.f. subp. *nigritana*, *Borassus aethiopum* Mart., *Chrysobalanus icaco* L. subsp. *icaco*, *Fegimanra africana* (Oliv.) Pierre, *Manilkara obovata* J.H. Hemsl., *Syzygium guineense* (Willd.) DC. subsp. *Guineense* (Miabangana et al., 2017). The undergrowth is dominated by: *Baphia leptostemma* Baill. Subsp. *leptostemma*, *C. dybowskii* Hutch. *Eugenia klaineana* (Pierre) Engl. *E. sumbensis* Greves, *Psychotria peduncularis* (Salisb.) Steyrm (Miabangana et al., 2017).

Its roots with high aromatic smell are highly used as masticatory and in aqueous soaking for the sexual

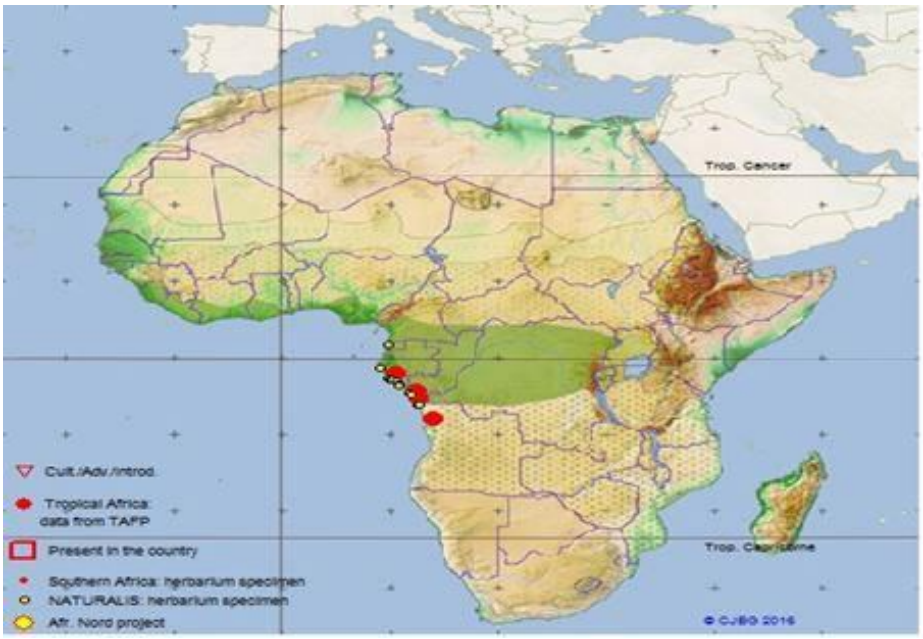

Figure 1. Geographical distribution of *C. dybowskii* Hutch. (Lebrun and Stork, 1991-2012).

asthenia and the pelvic pains treatment and also as a diuretic. The plant is threatened with the extinction due to its use as a consequence of its intensive marketing in the markets and bars in Brazzaville, Pointe-Noire and Kinshasa.

Previous studies focused on essential oils and isolated compounds made it possible to highlight some pharmacological properties on other *Croton*-type species: toxicologic (Fontenelle et al., 2008), antimicrobial (Simionatto et al., 2007; Baccelli et al., 2007; Kerntopf et al., 2008; Fontenelle et al., 2008; Daouda, 2015), molluscicide (Baccelli et al., 2007), antihypertensive and vasodilator (Guerrero et al., 2001), vasorelaxant (Daouda, 2015), cytotoxic and antitumor activity (Morales et al., 2005; Roenqsumran et al., 2002; Sandoval et al., 2002; Bezerra et al., 2009; Compagnane et al., 2010; Deore et al., 2009; Suganya et al., 2012), antioxidant activity (Sakina et al., 2016), insecticide, anti-microbial, anthelminthic, antimalarial, anti-inflammatory and leishmanicide (El Babili, 1997).

The chemical composition of the essential oils of other *Croton*-type species of various origins has already been carried out and the major constituents of its various tree species have been identified: β-caryophyllene (16.7%), germacrene D (14.7%) and borneol (8.3%) of *C. bonplandianus* (Joshi et al., 2014), (E)-caryophyllene (31.75%), germacrene-D (22.57%) and α-humulène (7.42%) of *C. hirtus* (Turiel et al., 2013), β-pinene (16.9%), α-pinene (16.5%), curzerene (12.8%) and germacrene D (9.0%) of *C. draconoides* (Daouda. 2015), sesquicineole (23.0%), dehydro-sesquicineole (13.8%), β-caryophyllene (7.9%), β-bisabolol (5.0%), germacrene D (4.2%) and β-elemene (4.1%) of *C. urucurana* (Daouda. 2015), β-phellandrene (18.2%), (E)-β-guaiene (16.5%), α-pinene (10.4%), (Z)-β-guaiene (15.9%) and (E)-caryophyllene (16.21%) of *C. sonderianus* (Giuliane et al., 2017), *(*E)-anethol (88.5%) of *C. zehntneri* (Giuliane et al., 2017), bicyclogermacrene (48.9%), β-caryophyllene (14.%) and germacrene D (12.6%) of *C. isabelli* (Giuliane et al., 2017), terpinen-4-ol (13.6%), β-caryophyllene (11.5%) and germacrene D (7.6%) of *C. pallidulus* (Giuliane et al., 2017), β-pinene (39.0%) and β-caryophyllene (8.1%) of *C. ericoides* (Giuliane et al., 2017), cymene (13.80%), 1.8 cineole (27.07%) and α-terpineol (6.87%) of *C. zambesicus*, (Sakina et al., 2016), α-pinene (20,98%), sabinene (11,21%) and bicyclogermacrene (28%) of *C. argyrophylloides* (De Souza et al., 2017), α-pinene, β-pinene (10%), (16,5%), (E)-caryophyllene (25,3%) and bicyclogermacrene (30.1%) of *C. sincorensis*, (De Souza et al., 2017), δ-elemene (12,8%) and β-elemene (22,2%) of *C. jacobinensis* (De Souza et al., 2017), bicyclogermacrene (30.0%), spathulenol (12.0%) and sabinene (11.0%) of *C. argyrophylloides* (Giuliane et al., 2017).

To the best of our knowledge, no chemical composition study of the essential oil of this plant has been carried out yet. Our objective in this present work is to study the chemical composition of the essential oil roots from *C. dibowskii*. Hutch and to evaluate its antioxidant power.

MATERIALS AND METHODS

Vegetable material

Croton dibowskii Hutch roots have been harvested in Congo-

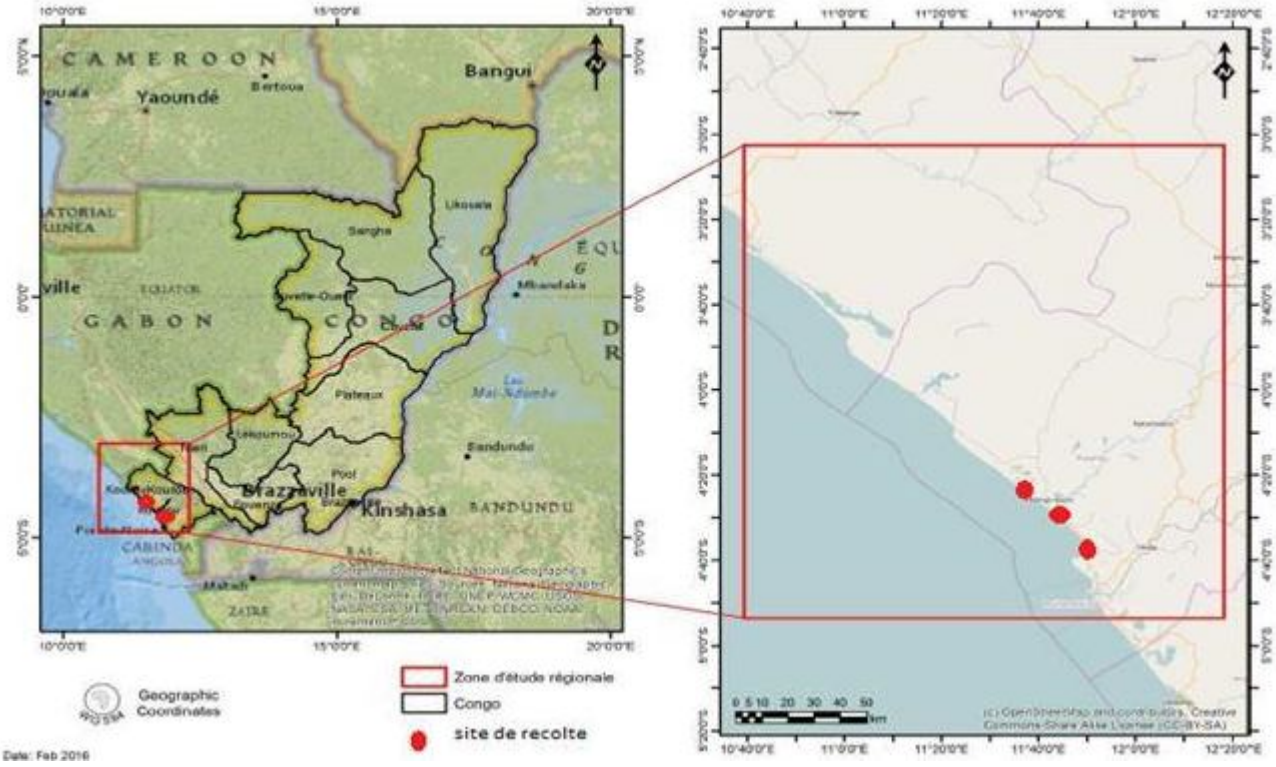

Figure 2. Map of the study area.

Brazzaville in the administrative department of Kouilou in Pointe Indienne (Figure 2) on the basis of the following map reference: S4 40.244 E11 48.861, Alt : -14 m. The different organs of the plant (Figure 3) have been compared with those of National Herbarium of the National Institute of Research in Exact and Natural Sciences (IRSEN) under number 188 (Miabangana et al., 2017).

The roots of the plant were dried at room temperature, for about a week. The dried vegetable was ground with an IKA-WERKE Gmbh-CO-KG, D-79219, Staufen-type device, with a sieve of granulometry 0.25 mm.

Method of production of the essential oil

The essential oil was obtained by vapohydrodistillation using Clevenger type apparatus (Clevenger, 1928). Each time, 300 g of vegetable material, consisting either only roots or stems, leaves and young flowers were placed in a flask with 500 ml of water and subjected to distillation. The organic phase from the distillation was separated from the aqueous phase by extraction with diethyl ether. The organic phase thus obtained was dried over anhydrous sodium sulfate to remove traces of water and the essential oil was recovered after evaporation of the diethyl ether and then move on to the chromatographic analysis immediately.

Analysis by gas chromatography (GC)

The quantitative analysis of the essential oil was carried out using an Agilent gas chromatograph model 6890 equipped with a DB5 column (20 m × 0.18 mm; 0.18 µm). The oven temperature was programmed to 50°C for 3.2 min, then heated to 300°C at a rate of 10°C/min. The temperatures of the injector and the flame ionization detector (FID) were maintained at 280°C. The essential oils were diluted in acetone to 3.5% (v/v) and injected in split mode (1/60); hydrogen was used as the carrier gas (1 ml/min), and the injection volume was 1 µl. At the same time, a solution of *n*-alkanes (C8-C30) was analyzed under the same conditions to calculate retention indices (RI) using the Van den Dool and Kratz equation (Van Del Dool and Kratz, 1963). The relative concentrations of the compounds were calculated from the peak area obtained by gas chromatography without using correction factors.

Analysis by coupling gas chromatography and mass spectrometry (GC-MS)

Qualitative analysis was performed using an Agilent gas chromatograph model 7890 coupled to a Agilent mass spectrometer model 5975 equipped with a DB5 MS column (20 m × 0.18 mm; 0.18 µm). The oven temperature was 50°C and remained constant for 3.2 min; then, it was increased to 300°C at a rate of 8°C/min. The injector temperature was 280°C. Ionization was obtained by electron impact at 70 eV, and the electron multiplier was maintained at 2200 eV. The temperature of the ion source was 230°C. Mass spectral data were acquired in the scan mode in the range m/z 33–450. The flow of carrier gas (helium) was set at 0.9 ml/min. The identification of the compounds was made by comparison of their mass spectra and RI with those of libraries such as Adams (2012), NIST (2008), König et al. (2001) and those of laboratory.

Determination of the antioxidant activity with 2.2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

Radical scavenging using DPPH radicals is the main mechanism which antioxidants act in food. The DPPH method as summarized

a b

Figure 3. Different organs of *Croton dybowskii* Hutch. (a) Roots, (b) small tree in the inshore thicket.

below was introduced nearly 50 years ago by Blois (1958).

The free radical-scavenging activity of extracts was measured by 2.2-diphenyl-2-picrylhydrazyl (DPPH). A solution of DPPH in methanol (24 µg/ml) was prepared and 2 ml of this solution was added to 50 µl of extracts solution in methanol at different concentrations (250, 500, 1000 and 2000 µg/ml). Then, the absorbance was measured at 517 nm in a spectrophotometer. In the original method, a reaction time of 30 min was recommended. Percent inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994). Scavenging effect (%) = $[(A_0-A_1)/A_0] \times 100$, where A_0 was the absorbance of the control sample (without essential oil) and A_1 was the absorbance in the presence of the sample.

RESULTS AND DISCUSSION

Chemical composition

The extraction gave yellow essential oil which yielded 0.4%. Its chemical essential, reported in Table 1, showed 53 identified compounds representing 90.8% of the chemical composition of the essential oil. The chromatographic profile remains dominated, for the most part, by cyperene (15.1%), borneol (12.5%), (6.9)-guaaiadiene (8.2%), α-muurolene (7.9%) and 1.8-cineol (6.7%). This essential oil is generally characterized by its high content in sesquiterpene hydrocarbons and oxygenated monoterpenes, which represent respectively 57.4 and 23.8%, that is, more than half of the total chemical composition of the essential oil. On the other hand, monoterpene hydrocarbons and oxygenated sesquiterpenes represent the smallest proportion that is 5.0 and 3.0%.

In addition, we noted the presence of epi-13-manool oxide with a content of 0.9%, corresponding to the only diterpenic compound present in the essential oil. It is appropriate to emphasize that the unidentified compounds represent 9.8% of the total chemical composition of the essential oil. Taken individually, their content does not exceed 3.0%. This chemical composition is close in general to the *Croton*-type essential oils (Joshi et al., 2014; Giuliane et al., 2017) by its richness in sesquiterpene hydrocarbons compounds but different from the latter by the individual content in cyperene (15.1%) and (6.9)-guaiadiene (8.2%), sesquiterpene hydrocarbons have never been reported in these latter. It is all the more different, by its quite high content in borneol (12.5%), compounds reported in some species (Joshi et al., 2014; Giuliane et al., 2017) of its kind but with smallest content proportion.

This essential oil is totally different from the oils of *C. zehntneri* (Giuliane et al., 2017), for its richness in (E)-anethol (88.5%), of *C. zambesicus* (Sakina et al., 2016), for its specific richness in aromatic monoterpene (cymene at 13.80%) and oxygenated (1.8 cineole at 27.07%) and l'α-terpineol at 6.87%) and of *C. hirtus*, *C. urucurana*, *C. isabelli, C. argyrophylloides*, *C. jacobinensis* for their specific richness in sesquiterpenic compounds (Turiel et al., 2013; Daouda, 2015; Giuliane et al., 2017; De Souza et al., 2017).

Antioxidant activity

The antioxidant activity of the *C. dybowskii* Hutch essential oil in the relation to DPPH• radical was estimated using spectrophotometer on watching the reduction of this radical which is accompanied by its transition from violet to (DPPH•) to yellow (DPPH-H) measurable at 517 nm.

The results of the antioxidant activity of the essential oil of *C. dybowskii* Hutch presented in Figure 3 show inhibition of 35% at the highest concentration (2000 µg/ml) with inhibitory concentration of 50% estimated at 3000 µg/ml. This power to reduce the free radicals observed in the present study could be explained by its chemical composition dominated by the cyperene (15.1%), borneol (12.5%), (6.9)-guaiadiene (8.2%), α-muurolene (7.9%) and 1.8-cineol (6.7%). This

Table 1. Chemical composition of the essential oil from *C. dybowskii* Hutch roots.

No. of order	RI	Constituents (*)	%
01	933	α-pinene	1.1
02	950	camphene	0.1
03	973	sabinene	0.1
04	980	β-pinene	2.2
05	1025	o-cymene	1.1
06	1029	Limonene	0.1
07	1030	1.8-cineol	**6.7**
08	1060	γ-terpinolene	0.1
11	1068	sabinene cis-hydrate	0.1
09	1089	Terpinolene	0.1
10	1096	Linalool	0.1
12	1146	Camphor	0.1
13	1150	hydrate camphene	0.1
14	1166	Borneol	**12.5**
15	1185	terpinen-4-ol	1.3
16	1196	α-terpineol	2.4
17	1217	Trans-carveol	0.2
18	1245	carvacrol methyl ether	0.4
19	1294	undecanone (2-)	0.1
21	1370	guaiadiene (6.9)-	**8.2**
22	1376	Isoledene	0.2
23	1377	α-copaene	1.5
24	1381	β-patchoulene	0.6
25	1391	β-elemene	0.7
26	1392	Sativene	0.8
27	1399	Cyperene	**15.1**
28	1408	amorpha –4.7(11)-diene	0.9
29	1418	α-santalene	0.8
30	1420	α-barbatene	1.7
31	1423	β-cedrene	1.7
32	1431	cis-thujopsene	1.3
33	1441	Aromadendrene	1.2
34	1453	NI	1.2
35	1460	allo aroamadendrene	0.4
36	1477	trans-cadina-1(6), 4-diene	0.2
37	1492	cis-β-guaiene	2.4
38	1493	δ-selinene	1.6
39	1496	Valencene	0.6
40	1497	Viridiflorene	0.5
41	1500	α-muurolene	**7.9**
42	1506	β-bisabolene	0.5
43	1509	Cuparene	0.5
44	1512	NI	1.1
45	1523	β–sesquiphelandrene	0.2
46	1539	α-cadinene	**7.5**

Table 1. Continues.

47	1530	NI	2.6
48	1555	NI	3.0
49	1559	NI	1.9
50	1593	Viridiflorol	2.6
51	1675	valeranone	0.4
52	1763	Aristolene	0.4
53	2057	epi-13-manool oxide	0.9
		Identified compounds	100
		Monoterpene hydrocarbons	4.9
		oxygenated monoterpenes	23.8
	Total	Sesquiterpene hydrocarbons	57.4
		oxygenated sesquiterpenes	3.0
		oxygenated diterpene	0.9
		Unidentified compounds	9.8

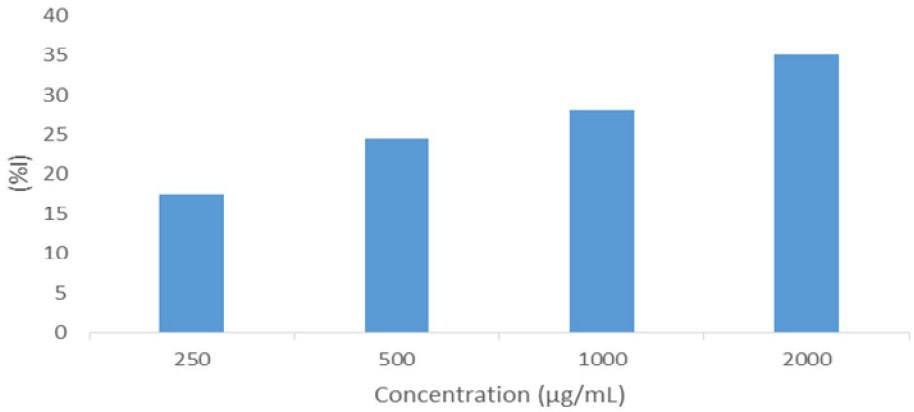

Figure 4. Radical scavenging activity of DPPH by the essential oil.

antioxidant activity is comparable to that of Sakina and al., (2016) on *C. zambesicus*. In fact, these authors had inhibitory concentration of 50% at 4200 to 3000 µg/ml estimated in the present study. They explained the activity of *C. zambesicus* by the presence of cymene (aromatic compound) identified at 13.80% and at 1.8 cineole and l'α-terpineol two oxygenated monoterpenes identified at 27.07% and 6.87% in the plant respectively. The latter used a dilution technique on 96-well plate (Molyneux, 2004). (Figure 4).

CONCLUSION

The chemical composition of the *C. dybowskii* essential oil showed a dominance of sesquiterpenic hydrocarbons compounds: cyperene (15.1%), (6.9)-Guaiadiene (8.2%), α-cadinène (7.5%) and α-muurolene (7.9%) followed by monoterpenic oxygenated compounds: borneol (12.5%), and 1.8-cineol (6.7%). In addition, we note the presence of epi-13-manool oxide with a content of 0.9% corresponding to the only diterpenic compound present in the essential oil. This chemical composition is close to essential oils *Croton*-type species (*C. bonplandianus* and *C pallidulus*) by its richness combined in sesquiterpene hydrocarbons and oxygenated monoterpnes and differs from the other *Croton*-type species (*C. zehntneri*, *hirtus*, *C. urucurana*, *C. isabelli* and de *C. argyrophylloides*) for their specific richness in (E)-anethol and in sesquiterpenic compounds. The antioxidant activity on the DPPH of the C*. dybowskii* Hutch essential oil showed a very low inhibition, not exceeding 35% at the concentration of 2000 µg/ml, this activity is comparable to some *Croton*-type species.

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