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Physico-chemical characterization and chemical profile of *Curcuma mangga* (Valeton et Zijp) essential oils acclimated in Congo-Brazzaville

Kévin Bikindou[1,2]*, Ernest Bitemou[1,2], Ghislaine Boungou-Tsona[1,2], Houzel-Mampouty Bounkosso[1,2], Aubin Nestor Loumouamou[1,2], Pierre Chalard[3,4], and Gilles Figueredo[5]

[1]Equipe Pluridisciplinaire de Recherche en Alimentation et Nutrition (EPRAN), Faculté des Sciences et Techniques, Université Marien Ngouabi, BP 389, Brazzaville, Congo.
[2]Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN), Cité Scientifique, BP 2400, Brazzaville-Congo.
[3]SIGMA Clermont, Université Clermont Auvergne, BP 10448, F-63000 Clermont-Ferrand, France.
[4]Institut de Chimie de Clermont-Ferrand, CNRS, UMR 6296, F-63178 Aubière, France.
[5]Laboratoire d'analyse des Extraits végétaux et des Arômes (LEXVA), Biopôle, Clermont-Limagne, Saint-Beauzire, France.

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ABSTRACT

This research aims to determine the chemical profile of the essential oil of *Curcuma mangga* (*C. mangga*). Samples of the essential oils, obtained by steam distillation of the harvested plant material (leaves and rhizomes) at four sites and during three years, were analyzed by GC-FID and GC-MS. The physicochemical characteristics of the volatile extracts, such as the relative density, the acid index, the ester index and the rotary power, were determined according to AFNOR standards. Analysis of the chemical composition shows that the essential oil of the leaves contains mainly ar-curcumene (14.42 to 32.08%), ɑ-zingiberene (3.5 to 16, 79%), β-sesquiphellandrene (5.48 to 14.07%), 1,8-cineole (traces-23.46%) and, to a lesser extent, β-bisabolene (3.25 to 9.11%). The rhizomes are rich in ɑ-zingiberene (11.56 to 33.28%), in β-sesquiphellandrene (14.24 to 20.85%), in curzerenone (traces-12.65%) and in camphor (0.61 to 16.44%). It appears that the chemical profiles with predominant of ar-curcumene for the leaves and predominant of ɑ-zingiberene and β-sesquiphellandrene for the rhizomes, are different from those identified in other countries.

Keywords: *Curcuma mangga*, chemical profile, ar-curcumene, ɑ-zingiberene, β-sesquiphellandrene, 1,8-cineole, camphor, curzerenone.

*Corresponding author. E-mail: bikindou.kevin@yahoo.fr.

INTRODUCTION

The genus *Curcuma* (family of Zingiberaceae), which includes a hundred species, is best known for being an essential source of coloring and flavoring agents in Asian and African cuisines (Leong-Skornikova and Newman, 2015). Several species of *Curcuma* are also used in traditional medicine to treat pneumonia, bronchial conditions, diarrhea, dysentery, abscesses and insect bites (Akarchariya et al., 2017; Chuakul and Boonpleng, 2003; Basaka et al., 2010).

Phytochemical studies on essential oils of *Curcuma* species have focused on the rhizomes and have led to the identification of monoterpenes and sesquiterpenes as major components of these oils. The rhizomes of *C. aerugunosa* cultivated in Thailand are rich in (i) germacrone (23.5%), curzerenone (11.8%) and 1,8-cineole (10.9%) (Theanphong et al., 2015); (ii) 1,8-cineole (22.7%), germacrone (17.7%), furanodiene (11.4%), and β-pinene (8%) (Srivilai et al., 2018). Those from Malaysia mainly contain (i) 1,8-cineole (23.2%) and curzerenone (28.4%) (Jantan et al., 1999); (ii)

curzerenone (24.6%), 1,8-cineole (11%), camphor (10.6%), zedoarol (6.3%), isocurcumenol (5.8%), curcumenol (5.6%), and furanogermenone (5.5%) (Sirat et al., 1998).

C. amada cultivated in India mainly contains more than 80% myrcene (Singh *et al*., 2002; Padalia et al., 2013; Choudhury et al., 1996a). But also (i) (Z) -β-Farnesene (21.9%), guaia-6.9-diene (19.8%), ɑ -longipinene (14.8%), ɑ-guaiene (14.5%), and camphor (5.5%) (Mustafa et al., 2005); (ii) (E) -hydroocimene (15.9%), (Z)-hydroocimene (14.2%), myrcene (14.9%), and linalool (13.4%) (Rao et al., 1989); (iii) ar-curcumene (28.1%), β-curcumene (11.2%), camphor (11.2%), curzerenone (7.1%), and 1.8- cineole (6%) (Srivastava et al., 2001).

This great variability of the chemical composition of the *Curcuma* species essential oils also results in a wide diversity of pharmacological properties attributed to these oils, in particular anti-inflammatory, anti-cancer, anti-proliferative, hypocholesterolemic, anti-diabetic, anti-hepatotoxic, anti-diarrheal, diuretic, anti-rheumatic , hypotensive properties, antioxidant, antimicrobial, antiviral, insecticidal, larvicidal, antivenom, antithrombotic, antityrosinase and cyclooxygenase-1 properties (Sikha et al., 2015; Afzal et al., 2013; Krup et al., 2013; Herath et al., 2017; Chen et al., 2008; Reanmongkol et al., 2006; Wilson et al., 2005; Angel et al., 2014; Mau et al., 2003).

C. mangga grows wildly in several regions of Congo Brazzaville. Locally, some populations use its leaves as wrapping leaves when preparing fish smother; the leaves bringing a spicy flavor. Considering the great chemical variability of essential oils of *Curcuma* species, the objective of the study is to characterize the chemical profile of essential oil of *C. mangga* acclimated in Congo Brazzaville.

MATERIALS AND METHODS

Plant material

The plant material consists of leaves and rhizomes of *C. mangga* harvested in the “plateau des cataractes” (Pool department) in Congo-Brazzaville. This is a transition zone between the southern and the northern part of Congo, limited to the north by the “plateaux Batéké”, to the southeast of Congo river and DRC, to the west by the Ndouo (Niari) and to the northwest by the foothills of Chaillu massif. Pool department has a Sudano-Guinean climate characteristic of the lower Congo comprising: (i) a long dry season lasting 4 to 5 months, coinciding with a temperature and water vapor tension minimum, linked to the cold Benguela Current flowing along the coast of Angola and the lower Congo, and (ii) a long rainy season but with reduced rainfall in January and February, a period called the “small dry season”, which is of some agronomic importance (Bikindou, 2017).

The samples were collected for three years and at four locations: Brazzaville (BZ), Loukoko (LK), Loulombo (LL) and Mindouli (MID). The sites of Loukoko, Mindouli and Loulombo are located respectively 100, 135 and 177 km from Brazzaville (Figure 1).

After collection, the samples were authenticated by the botanists of the National Research Institute for Exact and Natural Sciences (IRSEN) where the specimens were deposited for archives number 1423. The plant material was then dried out of direct sunlight and in an airy place (Figure 2).

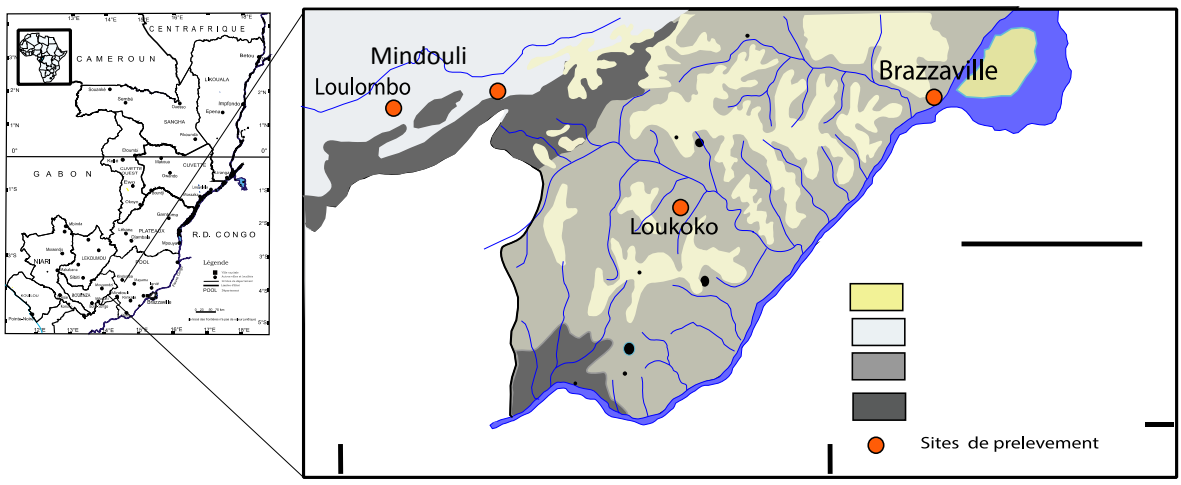

Figure 1. Cataract plateau area and harvest sites.

Essential oil extraction

Before distilling the plant material, the leaves were dried in the shade for 7 days while the rhizomes were first ground and then dried in the shade for 10 days. Steam distillation was carried out using a Clevenger type apparatus (Clevenger, 1928). Each time, 300 g of plant material consisting of either leaves or rhizomes, were placed in the 1 L tank which overcomes the 1 L flask containing 500 ml of water. Distillation was carried out for 6 hours. The organic phase resulting 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

A B

Figure 2. Different organs of *Curcuma mangga* (Valeton et Zijp). (A) leaves, (B) rhizomes.

traces of water and the essential oil was recovered after evaporation of the diethyl ether.

Determination of physico-chemical characteristics

The physicochemical characteristics such as the acid index (Ia), the ester index (Ie) the refractive index (η), the relative density D_{20} and the rotary power $[\alpha]^{D}_{25}$ have been determined according to AFNOR standards (AFNOR, 2000).

Gas chromatography (GC-FID)

The quantitative analysis of essential oils was carried out by an Agilent model 6890 chromatograph equipped with a DB5 column (20 m × 0.18 mm × 0.18 µm). The oven temperature has been programmed from 50°C for 3.2 min, then heat to 300°C at a speed of 10°C/min. The temperature of the injector and the flame ionization detector (FID) were maintained at 280°C. The essential oils were diluted in acetone at 3.5% (v/v) and injected in mode fractionated (1/60), hydrogen was used as carrier gas (1 ml/min), 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 the retention indices (RI) with the equation of Van den Dool and Kratz (1963). The relative concentrations of the compounds were calculated from the area of the peak obtained by gas chromatography without using correction factors.

Gas chromatography coupled to mass spectrometry (GC-MS)

The qualitative analysis was carried out using a gas chromatograph model Agilent 7890 coupled to a mass spectrometer model Agilent 5975, equipped with a DB5 column (20 m × 0.18 mm × 0.18 µm). The oven temperature is 50°C and remains constant for 3.2 min, then rises to 300°C at a speed of 8°C per minute, the temperature of the injector was 280°C. The ionization has was obtained by electronic impact at 70 eV and the electron multiplier was at 2200 eV. The temperature of the ion source was 230°C. Mass spectral data was acquired in scanning mode in the range m/z 33-450. The carrier gas flow rate (helium) is fixed at 0.9 milliliter per minute, the identification of the compounds was made by comparison of their spectra and their retention indices (RI) with those of libraries such as Adams (2012) and Nist (2008), and that made in the laboratory.

RESULTS AND DISCUSSION

Kinetics of extraction of essential oils

The determination of the essential oil content of a species first required the determination of the extraction time which made it possible to obtain the maximum amount of oil. Doing extraction kinetics thus allows us to know this duration. The extraction kinetics of the leaves and rhizomes were monitored for six (6) hours under the same conditions. The kinetic curve (Figure 3) shows that the essential oil content reaches a plateau after 5 hours of distillation, which suggests that from this time the maximum of oil is extracted.

These results made it possible to fix the duration of 6 hours for all the distillations made during this study. Compared to many essential oil species, the distillation time of Curcuma mangga which gives the most essential oil is high. On average, for many species, this duration is 3 hours (Bikindou, 2017).

Essential oil content

The data in Table 1 show that, for the samples collected at the four locations and during three harvest years, the average yield of the essential oil content of the leaves varies between 0.41% and 0.83%. Some years, it is observed yields above 1%, but this trend is not observed in other years.

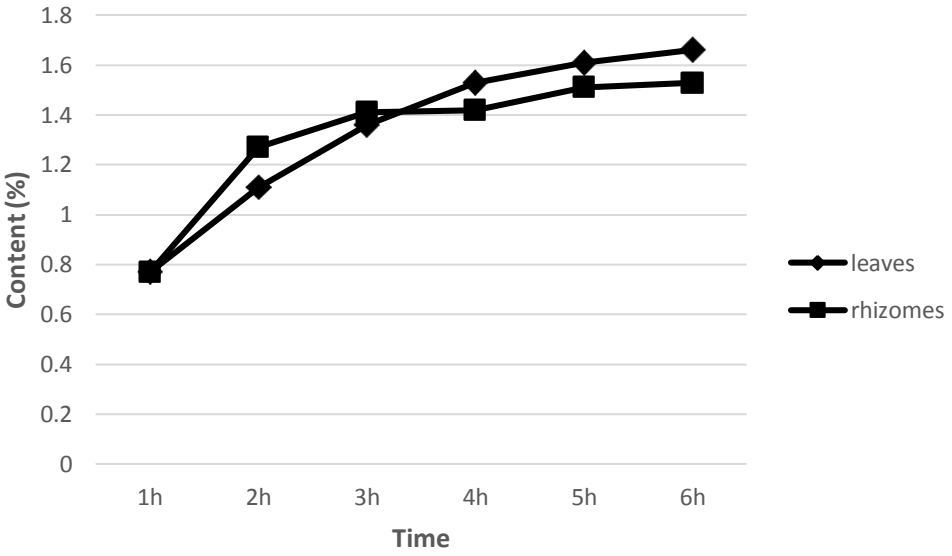

Figure 3. Essential oils extraction kinetics from leaves and rhizomes.

Table 1. Essential oil contents of the leaves and rhizomes samples.

Sites	MID	LL	BZ	LK
		Leaves (%)		
Year 1	0.15	0.18	0.76	0.59
Year 2	1.36	0.80	1.27	0.60
Year 3	0.17	0.24	0.43	0.75
Average ± σ	0.56 ± 0.69	0.41 ± 0.34	0.83 ± 0.42	0.65 ± 0.09
		Rhizomes (%)		
Year 1	0.82	0.17	0.16	0.11
Year 2	0.2	0.86	0.77	0.70
Year 3	1.68	1.34	1.03	1.64
Average ± σ	1.08 ± 0.52	0.79 ± 0.59	0.66 ± 0.45	0.82 ± 0.77

The essential oil content of the rhizomes is roughly in the same order of magnitude as that observed with the leaves. The average content varies between 0.66 and 1.08%. The results show that the leaves and rhizomes of *C. mangga* contain appreciable contents of essential oils and these contents do not exhibit significant variability depending on the harvesting area.

In general, and compared with other essential oil species (*Eucalyptus citriodora*, *Elionurus hensii*, etc.), the essential oil content of *C. mangga* is moderately low. *Eucalyptus citriodora*, for example, produces up to 6% (Loumouamou, 2008), similarly *Elionurus hensii* produces up to 3% (Bikindou, 2017).

Physico-chemical characteristics

The physicochemical characteristics determined are: the acid index (Ia), the ester index (Ie), the refractive index (Ir), the rotary power $[\alpha]^{D}_{25}$ and the density (D_{20}). Their values are given in Table 2.

Regardless of the harvest site, for the leaves as well as for the rhizomes, the values of the acid index of the essential oil are less than 1, which is in agreement with the AFNOR reference. The oil samples analyzed have a low ester content, the ester index is between 16.25 and 16.31 for the leaves, and between 16.24 and 16.32 for the rhizomes. The oils of the leaves and rhizomes have a refractive index between 1.470 and 1.500, as shown in the results in Table 2.

The data obtained on the rotary power show that this oil is sometimes levorotatory (value between -0.016 and -0.034 for certain leaf samples), sometimes dextrorotatory (value between +0.108 and +072). However, the samples of the rhizomes all exhibit a levorotatory character.

Table 2. Physicochemical characteristics of *C. mangga* essential oil.

Settings	Leaves				Rhizomes			
	MID	LL	BZV	LK	MID	LL	BZ	LK
Ia	0.57	*	0.55	0.51	0.59	*	0.51	0.53
Ie	16.25	*	16.27	16.31	16.24	*	16.32	16.29
Ir	1.47	1.48	1.48	1.47	1.47	1.48	1.47	1.50
$[\alpha]^{D}_{25}$	0.07	-0.03	0.10	-0.02	-0.05	-0.11	-0.12	-0.04
D_{20}	0.88	0.88	*	0.90	0.87	0.88	*	0.86

*Insufficient essential oil.

Chemical profile

The study of the chemical composition of essential oils from the leaves and rhizomes has identified around thirty chemical compounds.

Of all the samples, the essential oil of leaves (Table 3) is composed predominantly hydrocarbon sesquiterpenes the main ones are: ar-curcumene (14.42 to 32.08%), ɑ-zingiberene (3.5 to 16.79%), β-sesquiphellandrene (5.48 to 14.07%) and, to a lesser extent, β-bisabolene (3.25 to 9.11%). 1,8-cineole is the most important oxygenated monoterpene with contents varying between 2.22 and 23.46%, it's sometimes the majority constituent. Camphor (2.22 to 6.66%), another oxygenated monoterpene, is present at significant levels. This oil also contains hydrocarbon monoterpenes such as ɑ-pinene (traces-6.28%) and β-pinene (traces-5.13%).

Although studies of the chemical composition of the leaves of *C. mangga* are not found in the literature, the identified hydrocarbon sesquiterpenes are different from those found in the leaves of other *Curcuma* species. By way of comparison, the leaves of *C. aromatica* are rather rich in oxygenated monoterpenes such as 1,8-cineole, camphor and borneol (Singh et al., 2002; Bordoloi et al., 2017; Choudhury et al., 1996b; Al-Reza et al., 2010); those of *C. longa* are rich in a-phellandrene, terpinolene and p-cymene (Sindhu et al., 2011; Garg et al., 2002; Oguntimein et al., 1990; Priya et al., 2012; Pande and Chanotiya, 2006; Essien et al., 2015; Zaibunnisa et al., 2009).

Regarding the rhizomes (Table 4), on all four sites and during three years of harvesting, ɑ-zingiberene whose contents range between 11.56 and 33.28%, and β-sesquiphellandrene (14.24 to 20.85%) are the two most important major constituents. It should be noted that these two compounds are isomers (Figure 4). According to the samples, curzerenone (traces-12.65%), oxygenated sesquiterpene, and camphor (0.612 to 16.44%), oxygenated monoterpene, are the two other important compounds and can be considered as secondary major constituents, all like ar-curcumene (traces - 11.83%) which is however a hydrocarbon sesquiterpene.

Studies of *C. manga* rhizomes essential oil are few. The only results available are those obtained in Malaysia and which mainly present two profiles: (i) a chemical profile rich in caryophyllene oxide (18.7%) and caryophyllene (12.7%) (Kamazeri et al., 2012), (ii) another chemical profile rich in myrcene with contents of 81.4% (Jantan et al., 1999), 78.7% (Wong et al., 1999) and 46.5% (Wahab et al., 2011). It therefore appears that the chemical profiles identified to the Congo concerning *C. mangga* are different from those identified in Malaysia.

The treatment of the data by the ascending hierarchical classification (AHC), limiting itself to the chemical constituents whose content has reached at least 5% during the three years of the study (these constituents comprise on average 70% of all identified components), makes it possible to distinguish the dominant chemical profile of the leaves and that of the rhizomes (Figure 5).

For a dissimilarity level close to 0.08, the dendrogram mainly distinguishes two main classes:

- Class 1 groups all the individuals of the leaves, except one sample (BZ1Le)
- Class 2 consists of individuals from the rhizomes, except one sample (LK1Rh)

Classes 3 and 4 each consist of an "isolated" sample.

The results show that there is a certain homogeneity in the chemical composition of the leaf samples on the one hand and the rhizomes on the other hand, despite the harvests carried out at four different sites and during three years (Table 5). The essential oil of the leaves has a chemical profile rich mainly in ar-curcumene, while that of the rhizomes has a chemical profile rich mainly in ɑ-zingiberene and β-sesquiphellandrene. The chemical profile in 1.8-cineole remains to be confirmed by a more extensive study.

The abundance of these constituents in the oils of the leaves and rhizomes is also confirmed by the kinetic study (Figure 6). The distillation kinetics of the main volatile majority compounds in the leaves (Figure 6A) show that: (i) in the leaves, the distillation extract obtained in 1 hour is rich in 1,8-cineole. From 2 hours, the extract is enriched with ar-curcumene, ɑ-zingiberene and β-sesquiphellandrene and becomes depleted in 1,8-cineole (Figure 6A); (ii) In the rhizomes, the distillation

Table 3. Content of the main major compounds of the leaves essential oils.

Compounds	KI lit	KI exp	Content (%)										
			LK1Le*	LK2Le	MID1Le	MID2Le	MID3Le	BZ1Le	BZ2Le	BZ3Le	LL1Le	LL2Le	LL3Le
α-pinene	939	931	4.18	-	0.09	4.28	-	0.29	1.44	0.96	0.96	6.28	0.96
Camphene	946	947	1.98	-	0.05	2.01	-	1.98	0.18	0.13	0.47	1.96	0.54
Sabinene	975	970	0.28	-	-	0.26	-	0.41	2.97	-	0.13	0.64	-
β-pinene	979	975	3.58	-	0.16	3.39	-	1.17	1.17	-	1.16	5.13	1.16
Limonene	1029	1027	1.38	0.20	0.13	1.20	-	-	-	0.44	0.64	1.14	0.90
1,8-cineole	1031	1031	16.85	2.40	2.17	11.15	0.34	23.46	13.99	3.88	6.19	12.12	4.77
Cis-hydrate sabinene	1070	1069	0.68	-	-	-	-	-	1.12	-	0.08	1.20	-
Linalool	1099	1097	1.18	0.34	1.51	0.71	-	1.68	-	0.62	1.23	-	0.52
Camphor	1146	1148	6.61	1.95	5.86	5.85	0.65	6.66	5.00	3.45	4.41	5.02	3.45
Isoborneol	1160	1165	1.49	0.38	1.46	1.28	0.61	0.42	1.27	0.52	0.95	0.57	0.52
Borneol	1169	1174	0.49	-	1.41	0.62	-	0.09	0.81	-	0.69	0.27	-
α-terpineol	1188	1196	0.78	-	1.25	-	0.67	0.12	-	-	0.69	-	-
β-elemene	1390	1389	0.27	-	0.41	0.49	0.51	-	0.36	0.78	0.85	0.37	-
Sesquithujene	1405	1402	0.16	0.63	0.60	0.24	0.61	0.08	0.38	0.41	0.42	0.79	0.71
β-caryophyllene	1419	1421	0.36	0.57	0.74	0.50	0.67	0.18	0.42	0.89	0.97	0.40	0.54
γ-elemene	1431	1428	-	0.12	0.09	0.17	0.09	0.27	0.15	0.12	0.20	0.21	0.29
Trans-β Bergamotene	1434	1432	0.11	0.63	0.18	0.15	0.19	0.03	0.16	0.16	0.16	0.62	0.66
(E)-β farnesene	1454	1450	0.32	0.72	0.25	0.32	0.77	0.10	0.45	0.72	0.34	0.16	-
ar-curcumene	1480	1482	21.00	18.52	26.78	19.94	26.86	14.42	21.49	18.52	16.53	21.49	29.24
α-zingiberene	1493	1495	3.55	12.97	10.35	7.92	16.80	14.80	5.75	12.97	15.87	5.75	10.96
β-bisabolene	1505	1507	4.40	5.65	6.06	4.84	8.35	3.25	5.21	5.65	4.99	4.77	9.11
β-sesquiphellandrene	1522	1518	5.49	13.14	9.90	8.67	10.67	12.30	8.29	13.14	13.84	12.94	11.66
Cis-hydrate sesquisabinene	1544	1554	0.68	0.69	0.96	0.60	0.37	0.53	0.57	0.70	0.87	0.56	-
Germacrene-B	1561	1561	-	0.83	0.65	0.25	1.40	0.82	0.29	0.83	1.08	0.17	-
ar-turmerol	1583	1578	-	0.44	1.28	0.71	-	0.93	-	0.44	0.84	0.05	2.60
Caryophyllene oxide	1583	1585	1.66	1.19	2.16	1.01	2.06	0.68	1.46	1.19	1.04	0.99	2.19
Trans-hydrate sesquisabinene	1579	1590	-	1.94	1.53	0.18	0.97	0.80	-	1.99	1.67	0.67	1.09
Trans-β elemenone	1602	1601	-	3.73	1.98	0.25	2.89	0.39	0.33	4.12	3.76	0.71	0.69
Curzerenone	1606	1604	0.67	1.08	4.42	1.89	5.72	2.39	0.08	5.72	4.42	1.33	0.99
Germacrone	1696	1696	1.84	6.46	2.97	2.26	4.28	1.49	2.05	5.46	5.57	2.21	6.53
ar-curcumen-15-al	1713	1713	1.85	3.20	1.55	1.36	1.78	0.50	0.94	1.72	1.08	0.22	2.51
Total			**81.84**	**76.78**	**86.95**	**82.5**	**87.26**	**90.24**	**76.33**	**85.53**	**92.10**	**88.74**	**92.59**

* (1): year 1; (2): year 2; (3): year 3 (Except for the LK site, the harvest could not be done); Le: Leaves

Table 4. Content of the main major compounds of the rhizomes essential oils.

Compounds	KI lit	KI cal	Content (%)											
			LK1Rh	LK2Rh	LK3Rh	MID1Rh	MID2Rh	MID3Rh	BZ1Rh	BZ2Rh	BZ3Rh	LL1Rh	LL2Rh	LL3Rh
α-pinene	939	912	1.49	1.90	-	1.80	3.23	0.85	1.40	1.98	0.50	1.89	1.85	0.89
Camphene	946	928	1.91	1.19	-	1.06	1.85	0.55	-	1.31	0.21	1.45	1.18	0.69
Sabinene	970	973	0.09	-	-	0.14	-	-	-	-	-	0.14	-	-
β-pinene	979	975	6.94	4.99	1.41	5.02	5.62	2.39	5.11	4.92	1.63	4.25	4.60	2.40
Myrcene	990	987	0.25	-	-	0.18	-	-	0.74	-	-	0.26	-	-
Limonene	1029	1028	0.61	0.49	0.26	0.48	0.71	0.20	0.12	0.76	0.72	0.50	0.36	0.24
1,8-Cineole	1031	1030	0.90	0.54	0.15	0.44	0.38	0.17	0.14	0.55	-	0.75	0.19	0.49
Camphor	1146	1148	16.46	7.01	1.66	6.70	6.42	3.32	7.01	8.55	2.11	0.61	4.32	4.46
Isoborneol	1160	1165	4.17	2.19	0.73	2.08	2.19	1.35	0.09	2.70	0.86	7.14	1.61	1.76
Borneol	1169	1174	1.43	0.82	-	0.61	0.67	0.44	1.97	0.89	0.20	2.16	0.50	0.52
β-elemene	1390	1390	0.34	0.58	0.58	0.29	0.41	0.19	-	0.48	0.50	0.27	0.43	0.29
Sesquithujene	1391	1402	0.25	0.22	0.26	0.20	0.34	0.18	-	0.48	0.28	0.21	0.24	0.25
β-caryophyllene	1431	1430	0.23	0.28	0.55	0.25	0.20	0.12	-	0.24	0.23	0.21	0.29	0.16
γ-elemene	1434	1434	0.16	0.37	0.31	0.11	0.22	0.17	2.34	0.25	0.12	0.64	0.24	0.30
Sesquisabinene	1452	1455	-	0.50	0.61	0.14	0.61	0.23	0.11	0.51	0.53	-	0.41	0.36
γ-curcumene	1480	1483	-	5.97	0.62	0.04	5.27	0.60	0.14	5.71	0.67	4.69	4.61	0.80
ar-Curcumene	1480	1482	-	5.97	11.66	0.09	5.26	6.37	4.49	5.71	11.84	4.69	4.61	7.15
α-zingiberene	1493	1495	11.56	27.98	19.61	24.17	31.28	25.13	33.12	25.19	21.01	25.34	30.52	22.24
β-Bisabolone	1505	1507	3.52	4.26	5.15	3.43	4.53	4.10	4.09	3.92	5.60	3.47	3.38	3.92
β-sesquiphellandrene	1522	1525	14.25	17.79	19.26	16.87	20.86	16.12	16.77	18.04	20.39	16.77	17.70	15.45
Germacrene-B	1561	1562	0.62	0.43	1.17	0.70	0.52	0.34	0.56	0.62	0.73	0.55	0.47	0.48
Caryophyllene oxide	1583	1586	0.62	0.68	0.67	0.34	0.14	0.60	0.62	0.45	0.62	0.55	0.37	0.80
Sesquisabinene hydrate	1590	1593	0.42	0.71	1.22	0.55	0.54	1.37	1.27	1.28	1.43	1.41	0.89	1.73
Curzerenone	1606	1604	0.42	7.98	12.32	1.04	3.41	12.66	6.68	7.78	9.66	5.97	10.85	11.70
ND		1617	0.33	2.13	3.98	0.16	1.40	4.21	0.22	3.22	3.60	9.91	0.48	4.80
ND		1635	1.62	0.33	1.93	2.64	0.57	2.17	0.21	1.48	1.95	0.63	1.04	-
Germacrone	1693	1698	6.06	0.78	11.11	6.02	2.02	4.21	6.64	1.92	7.54	3.08	4.84	7.66
Total			**74.65**	**96.09**	**95.22**	**75.55**	**98.64**	**84.25**	**93.84**	**98.94**	**74.65**	**97.54**	**95.98**	**89.54**

extract contains significant contents of α-zingiberene and β-sesquiphellandrene from the first hour of distillation (Figure 6B). The concentration of α-zingiberene continues to increase to reach the maximum in the 5-hour extract.

The presence in large quantities of constituents such as 1,8-cineole, ar-curcumene or β-sesquiphellandrene, suggests that this essential oil has significant biological activity. Indeed, 1,8-cineole possesses strong antioxidant and anticarcinogenic activities. β-sesquiphellandrene demonstrated remarkable DPPH-scavenging activity. It showed anticancer potential when compared with curcumin and was cytotoxic to the mouse lymphocytic leukemia (L1210) cell line. ar-curcumene appears to be responsible for the anti-tumor effects of *C. zanthorrhiza* (Noura et al., 2018).

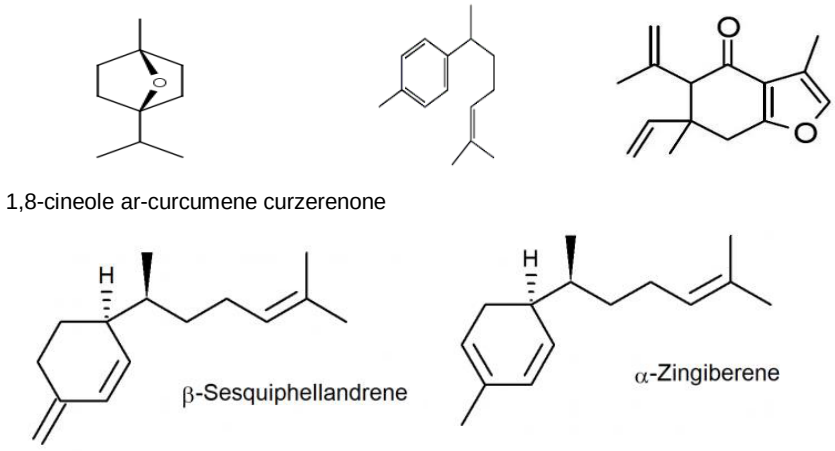

Figure 4. Structures of the main major compounds of *C. manga.*

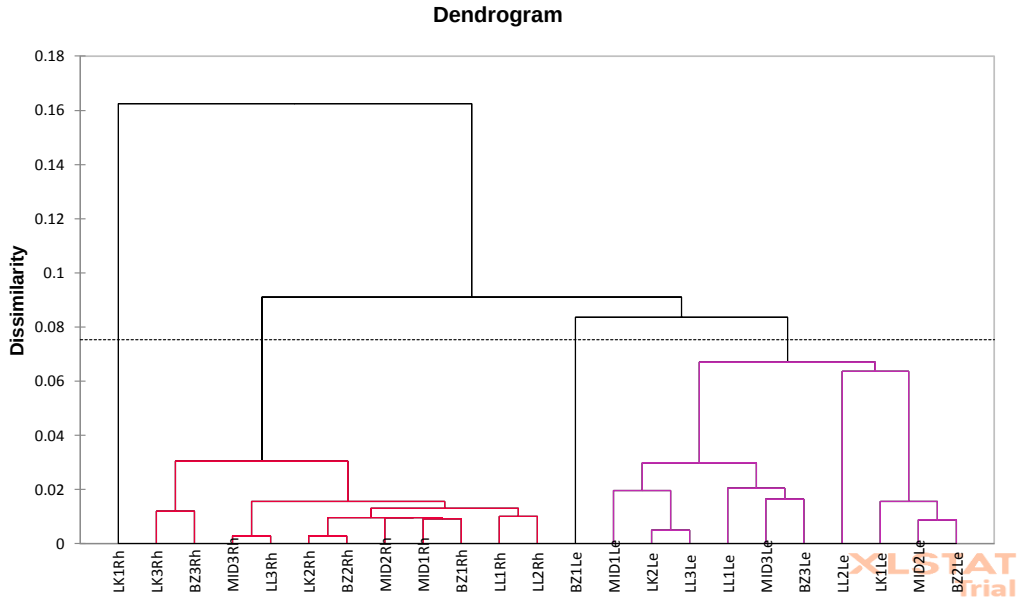

Figure 5. Dendrogram in AHC of 23 leaves and rhizomes samples.

Table 5. Distribution of samples by class generated by AHC.

Class	1	2	3	4
Samples	LK1Le, LK2Le MID1Le, MID2Le MID3Le, BZ2Le BZ3Le, LL1Le LL2Le, LL3Le	LK2Rh, LK3Rh MID1Rh, MID2Rh MID3Rh, BZ1Rh BZ2Rh, BZ3Rh LL1Rh, LL2Rh	LK1Rh	BZ1Le
1,8-cineole (%)	2.22 - 16.84	-	-	23.46%
Camphor (%)	2.22 - 6.66	0,61 - 8.55	16.44	6.66%
ar-curcumene (%)	14.42 - 32.08	Traces - 11.83	-	14.42
ɑ-zingiberene (%)	3.5 - 16.79	11.56 - 33.28	11.56	14.80
β-sesquiphellandrene (%)	5.48 - 14.07	14.24 - 20.85	14.24	12.30
Curzerenone (%)	0.66 - 5.72	0.42 - 12.32	6,67	2,39
Germacrone (%)	1.49 - 6.52	0.78 - 11.11	6,06	1,49

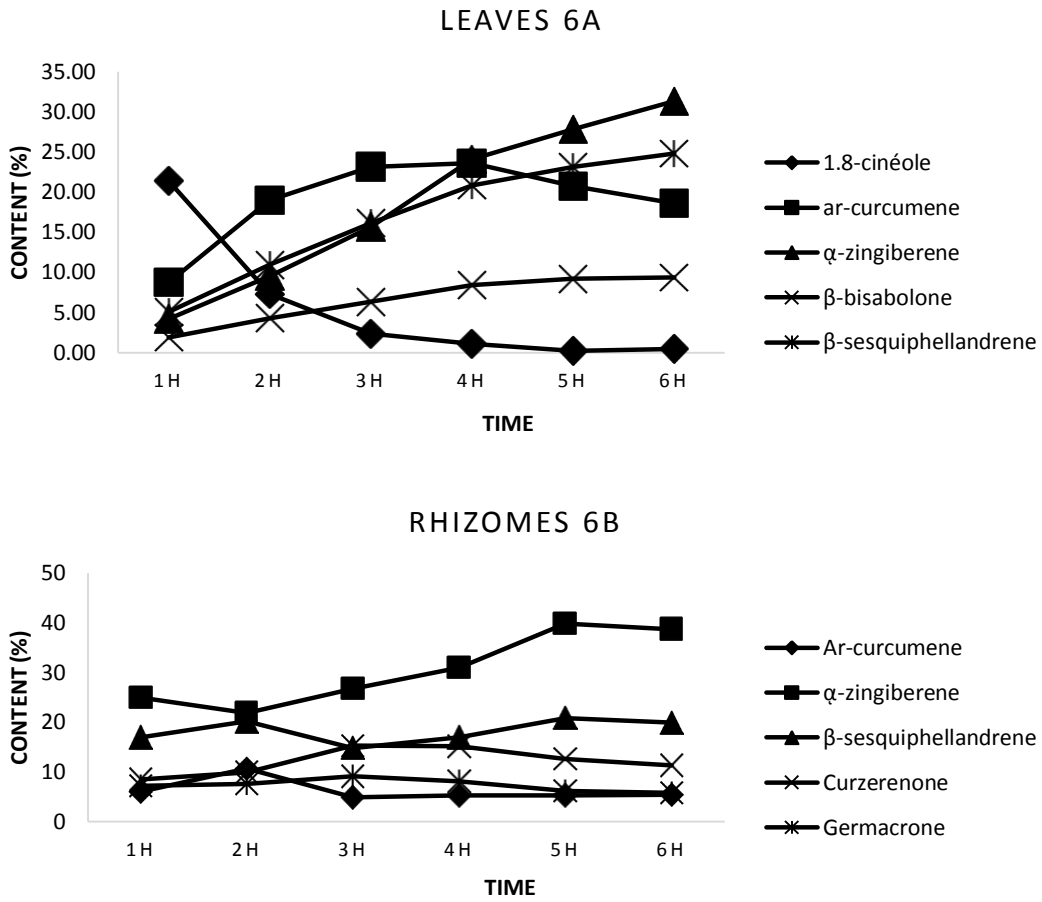

Figure 6. Distillation kinetics of the majority compounds of leaves (BZ2Le) and rhizomes (BZ2Rh).

CONCLUSION

The essential oil chemical profile of leaves is somewhat different from that of rhizomes, although qualitatively one found mainly ar-curcumene the α-zingiberene and the β-sesquiphellandrene in leaves and rhizomes. The ar-curcumene which is the main compound of the leaves is found at lower contents in the rhizomes. Conversely, α-zingiberene and β-sesquiphellandrene are high in the rhizomes and lower in the leaves. In addition, the leaf oil contains high levels of 1,8-cineole, (only traces in the rhizomes) while camphor and curzerenone are high levels in the rhizomes. The chemical profile of the leaves is predominantly ar-curcumene while that of the rhizomes is predominantly α-zingiberene and β-sesquiphellandrene.

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