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Physico-Chemical Attributes of Essential Oil from *Zingiber officinale* Roscoe and *Zingiber zerumbet* (L.) Smith Cultivars Grown in Togo

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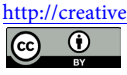

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Abstract

The present study was carried out to evaluate the quality of the rhizomes of *Zingiber officinale* and *Zingiber zerumbet* from different areas of Togo on the basis of their essential oil content and composition. The dry rhizomes were pulverised, hydrodistilled and essential oils were analysed using GC and GC/MS techniques. The results revealed that the essential oil yield of *Z. zerumbet* ranged from 0.75% to 1.00% and was mainly composed by Zerumbone (51.8% - 37.5%) and humulene (21.7% - 28.2%). *Z. officinale* essential oil yielded from 1.25% to 1.67%, with major components as zingiberene (33% - 39.9%); basibolene (12.7% - 16%), and sesquiphellandrene (13% - 14.7%).

Keywords

Zingiber officinale, *Zingiber zerumbet*, Rhizome, Essential Oil, Togo

1. Introduction

The genus Zingiber, belonging to the Zingiberaceae family, is represented by about 141 species among which are *Z. officinale* and *Z. zerumbet* [1]. Both species are perennial herbaceous monocotyledon, usually grown as annual in tropical and subtropical regions [2]. *Z. officinale* is well-known as ingredient for dishes in almost tropical and subtropical countries [3]. Fresh rhizome is used as for flavoring and taste in different foods during cooking and in formation of compounded aromas for flavoring confectionery, bakery products, condiments, sauces and carbonated beverages while the dried powder is used as spice [3]. *Z. officinale* has been widely used for centuries in traditional medicine for various

purposes, such as rhinitis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis [4]. The essential oil obtained from rhizomes has been reported to exhibit several biological activities, such as antimicrobial [5] [6] [7] [8], antioxidant [5], and antifungal [6] [9]. It contains a mixture of constituents such as monoterpenes, namely phellandrene, camphene, cineole, linalool, limonene, citral, geraniol, citronellol, borneol and sesquiterpenes, namely α-zingiberene, *ar*-curcumene, β-bisabolene, β-sesquiphellandrene, δ-cadinene, zingiberol and zingiberenol along with some aliphatic aldehydes and alcohols [8] [10] [11] [12] [13] [14]. As for *Zingiber zerumbet* (L.) Smith, it is widely cultivated in the tropics for its medicinal properties and as a marketable spice [15]. Among its uses are: treatment for stomachache, swelling, sores and loss of appetite and as an ingredient of several medical preparations used to treat ear inflammation and diarrhea in traditional medicine in many countries [16] [17]. Its rhizomes contain essential oil which is used as medicinal drugs, spices and raw materials for industry [18]. The essential oil is the mixture of terpenes namely zerumbone, α-caryophyllene and camphene [19], (Z)-citral, camphene, sabinene, zingiberene and lavandulyl acetate [20], zerumbone, α-humulene, β-selinene and (-)-caryophyllene oxide [21]. The composition of volatile oil is highly variable depending upon a variety of factors, in particular the geographical origin [22] [23] [24]. Hence, this study was undertaken to evaluate the chemical composition of essential oil from rhizomes of *Z. officinale* and *Z. zerumbet* cultivated along different parts of Togo.

2. Material and Method

2.1. Plant Material

Samples of the *Z. officinale*, *Z. zerumbet* species were collected directly from the main growing areas namely Kougnohou (N: 07.61469 E: 000.79146), Badou (N: 07.60491 E: 000.68892), Danyi Apéyémé (N: 07.16162 E: 000.69497), Kaniamboua (N: 08.364145 et E: 001.001571), Dalanda (N: 08.378180 E: 001.004719), Blitta-Tadjan (N: 08.198874 E: 0.796531) to ensure their origin (**Table 1**). They

Table 1. Sampling areas of *Z. officinale* and *Z. zerumbet* rhizomes.

Cultivar	Sampling areas	Number of samples collected
Z. officinale	Badou Danyi Apéyémé Kougnohou Blitta Tadjan Dalanda Kaniamboua	6
Z. zerumbet	Dalanda Kaniamboua	2
TOTAL		8

were then Taxonomically identified and authenticated in the Laboratoire de Botanique et d'Ecologie Vegetale of the Faculte des Sciences of the Universite de Lome. A voucher specimen was deposited in the herbarium division of the laboratory.

2.2. Preparation of Plant Material

The fresh rhizomes were washed thoroughly under running tap water to remove adhered soil and foreign matter. The clean rhizomes were cut into small pieces, dried under ventilated shelter for two weeks and subsequently pulverized into fine powder using a mechanical grinder. The powder was stored at room temperature (28˚C ± 2˚C) in an air tight container until it was used.

2.3. Extraction of Essential Oils

Fifty grams (50 g) of the fine powder of each material were introduced in the Clevenger-type apparatus with 0.5 liter of distilled water [10]. The mixture was put under heating at 100˚C using a hot plate-type IRS France and extracted for 2 hours. The essential oil which evaporates with the steam was condensed into a collecting separating funnel. The oil was collected without any treatment and stored at 4˚C in the dark bottle tightly closed. After each extraction process, the Clavenger apparatus was washed by vacuum extraction for at least 30 minutes and then the entire circuit was then rinsed with alcohol before run another sample. The yields of samples were averaged over three experiments and calculated based on dry weight of the rhizomes.

2.4. Moisture Content Measurement of the Rhizomes

The moisture content of the ginger rhizomes was determined according to ASAE Standard S358.2 [25]. The sample was dried in an electric oven at a temperature of 105˚C during 24 hours and weighed using a weighing balance. The moisture content of the sample in percent dry basis was calculated using Equation (1).

$$M_s(\%) = \frac{100(W_i - W_f)}{W_f} \quad (1)$$

where: M_s is the Moisture Content of Ginger rhizomes (in % dry basis), W_i is the Initial Mass of ginger rhizomes before oven drying (in grams) and W_f is the Final Mass of the rhizomes after oven drying (in grams).

2.5. Physicochemical Analysis of the Essential Oils

The extracted essential oils were evaluated for yield, refractive index and density according to the methods below.

2.6. Density Measurement

A volume of approximately five (5) ml of each essential oil sample was taken using the METTLER TOLEDO Dansito 30PX density meter after calibration with distilled water. The density values as well as the corresponding measurement

temperatures are displayed on the display of the device. The density was evaluated at 20˚C for this experiment.

2.7. Refractive Index Determination

The refractive index of the different essential oil samples was determined by refractometer HR-110N OPTIKA. One drop of the oil was dropped on the cell compartments of the instruments. The necessary adjustment was made and the result recorded when the lower part became darker [26].

2.8. Yield Determination

The yield which represents the quantity of essential oil extracted from plant materials was recorded as volume: weight ratio [27] [28] [29]

$$\text{Yield}(\%) = \frac{\text{weight of extract recovered}}{\text{weight of dried plant material}} \times 100$$

2.9. GC-Analysis of Essential Oils

2.9.1. GC/FID Analysis

The simple gas phase analysis was carried out by a Varian 3900 type chromatograph equipped with a flame ionization detector (FID), a non-polar DB-5 ms 30 m × 0.25 mm column; film thickness 0.25 µm df (J&W Scientific). The injector and the detector were maintained at 300˚C. The carrier gas used was helium with a flow rate of 1.6 mL/min. The sample (1 µl) was injected into the column with a split ratio of 1:150. Component separation was achieved following a linear temperature programmed from 60˚C to 180˚C with a gradient of 2˚C/min then increased to 280˚C at 9˚C/min and kept in isothermal at 280˚C for 10 minutes. Percentage of the constituents were calculated by electronic integration of FID peak areas.

2.9.2. GC/MS Analysis

Essential oils were analyzed on an Agilent gas chromatograph Model 6890, coupled to a Agilent MS model 5973, equipped with a DB5 MS column (30 m × 0.25 mm × 0.25 µm film thickness). Essential oils were diluted to 5% in dichloromethane and injected under the following conditions: injector temperature of 300˚C, injection volume 1 µL at a ratio of 1:80 (split mode), initial column temperature of 60˚C; heated gradually to 180˚C at a 2˚C/min rate; heated to 230˚C at a 5˚C/min rate, kept for 5 min. The carrier gas (helium) flow was set at 1.3 mL/min. The temperatures of the transfer line, ion source, and injector were 240˚C, 230˚C, and 300˚C, respectively. The MS works in electron impact mode at 70 eV; electron multiplier, 1500 V. The mass spectra were obtained at a range of mass/load ratio (m/z) 33 - 450 in scan mode with a solvent delay time of 3 min. The compounds were identified by comparison of their retention index (RI) obtained using homologous series of n-alkanes standards (C5 - C18) and their mass spectra were compared with the NIST2014 and Wiley2008 library and the literature [3] [30] [31].

3. Results and Discussion

3.1. Physico-Chemical Parameters of the *Z. officinale* and *Z. zerumbet* Cultivars

The physical parameters (moisture content, essential oil yield, density and refractive index) both for *Z. officinale* and *Z. zerumbet* rhizomes samples were found to varied according to the sampling area (Table 2).

The lowest moisture content of *Z. officinale* rhizomes was observed in samples from Danyi Apéyémé (79.48%) and the highest in samples from Badou (86.59%). The majority of samples (7/8) showed moisture content higher than 80%. The moisture content of studied samples is in similar range with the moisture content reported in *Z. officinale* rhizomes from Bangladesh (84.97% to 87.98%) [32] while it is higher than what reported in *Z. officinale* rhizomes from India (76.18% - 78.84%) [33]. The essential oil yield varied from 1.25% to 1.67% with the lowest yield obtained in the samples from Kougnohou and the highest yield in the samples from Kaniamboua. Samples from three localities (Kaniamboua, Danyi Apéyémé, Blitta-Tadjan) showed an essential oil yield higher or equal to 1.5%. Steam distillation of *Z. officinale* essential oil yields 0.2% to 3.0% according to the origin and the state of the rhizome (fresh or dry) [10] [30] [34] [35]. The density of essential oils ranged from 0.876 (Badou) to 0.897 (Blita-Tadjan) and the refractive index from 1.487 (Dalanda and Danyi Apéyémé) to 1.491 (Blitta-Tadjan). Very little variation was observed for the two parameters. The values of 0.877 to 0.883 for density and 1.4884 to 1.4918 for refractive index which are close to our findings were reported in *Z. officinale* rhizome essential oil samples from Madagascar [36].

Z. zerumbet samples showed moisture content higher than 85% (87.23% to 91.99% respectively for Dalanda and Kaniamboua samples) and an essential oil yield not exceeding 1% (0.75% to 1.00% respectively for the Dalanda and Kaniamboua samples). Crystals uniformly mixed with rhizome oils were observed,

Table 2. Physical parameters (moisture content, yield, density, refractive index) of *Z. officinale* and *Z. zerumbet* rhizomes.

Cultivars	Harvest locations	Rhizomes	Extracts		
		Moisture content (% w/w)	Yield (% w/w)	Density	Refractive index
Z. officinale	Dalanda	82.27	1.33	0.880	1.487
	Kaniamboua	85.46	1.67	0.882	1.489
	Danyi Apéyémé	79.48	1.50	0.881	1.487
	Badou	86.59	1.42	0.876	1.490
	Blitta-Tadjan	82.03	1.50	0.897	1.491
	Kougnohou	81.26	1.25	0.878	1.490
Z. zerumbet (L.)	Dalanda	87.23	0.75	ND	ND
	Kaniamboua	91.99	1.00	ND	ND

*ND: the crystallization of *Z. zerumbet* essential oil did not allow to do these measurements.

giving unsuitable properties to the extract for the determination of the density and the refractive index. Varied yields have been reported elsewhere, namely 0.3% to 0.4% in *Z. zerumbet* rhizomes from Reunion Island [37]; 0.39% to 0.65% in rhizomes from China [38] and 1.1% in rhizomes from Bangladesh [19].

In general, these variations are attributable to many factors such as topological and geographical location as well as maturity of rhizomes, harvesting time, genetic variation, extraction process [10] [22] [39] [40] [41]. Even if both *Z. officinale* and *Z. zerumbet* rhizome from Kaniamboua present best potential for essential oil production and commercialization, all sample match international requirement as soon as they yielded higher than 0.4% [42].

3.2. Aromatic Profile of the *Z. officinale* and *Z. zerumbet* Cultivars

The aromatic profile of the samples from the different sampling areas is presented in **Table 3** for *Z. officinale* and **Table 4** for *Z. Zerumbet*.

The GC/MS profile of essential oils of *Z. officinale* showed thirty-eight components accounting for 92.4%; 90.1%; 91.7%; 92.3%; 91.8% and 93.1% of dry rhizome essential oil samples from Dalanda, Kaniamboua, Danyi-Apéyémé, Badou, Blitta-Tadjan and Kougnohou respectively. Essential oil from all sampling areas showed high predominance of sesquiterpenes (69.9% to 80.2%) and monoterpenes (5.9% to 13%), while oxygenated monoterpenes (2.6% to 5.2%) and oxygenated sesquiterpenes (2% to 3.3%) were found in low level. The highest content of sesquiterpenes, monoterpenes, oxygenated monoterpenes and oxygenated sesquiterpenes were found respectively in the samples from Badou (80.2%), Danyi Apeyeme (13%), Kaniamboua (5.2%) and Dalanda (3.3%) while the lowest levels were found in samples from Kaniamboua (69.9%), Dalanda and Badou (5.9%), Danyi Apeyeme (2.6%) and Kaniamboua (2%).

Zingiberene was the major sesquiterpene identified in the essential oil of all the samples. The higher zingiberene content was registered in Badou samples (39.9%) followed by Danyi-Apéyémé (39.7%) and Blitta-Tadjan (39.2%) while the lower content was found in Kaniamboua samples (33%). Basibolene (12.7% to 16%) and sesquiphellandrene (13% to 14.7%) was the second highest component in all the rhizomes sampled. Basibolene content was highest in Badou samples (16.0%) while the lowest content was recorded in Kaniamboua samples (12.7%). Sesquiphellandrene content did not vary widely, and the highest was observed both in Badou and Blitta-Tadjan samples (4.7%) while the lowest was found in Kaniamboua samples (13.0). The other major sesquiterpene was α-curcumene which ranged from 5.8% (Dalanda) to 4.5% (Danyi-Apéyémé), but was absent in Blitta-Tadjan samples. The γ-murolene content varied from 1.0% (Kaniamboua) to 4.7% (Blitta-Tadjan) and was absent in Danyi-Apéyémé and Badou samples. The germacrene was also present in notable amount in all samples and varied from 1.6% (Kaniamboua) to 2.4% (Kougnohou). The β-farnesene content just get over 1% in Dalanda samples while it was below 1% in other samples.

Table 3. Aromatic profil of *Z. officinale* L. from six sampling areas in Togo.

Peak N°	Component	Sampling areas and content (%)						IR/MS	Identification
		Dalanda	Kaniamboua	Danyi-Apéyémé	Badou	Blitta-Tadjan	Kougnohou		
Monoterpenes		**5.9**	**13**	**8.6**	**5.9**	**7.4**	**7.3**		
1	*α*-pinene	0.5	1.2	0.7	0.4	0.6	0.7	932	MS, RI
2	camphene	1.9	4.6	2.8	1.8	2.6	2.9	948	MS, RI
3	*β*-pinène	0.0	0.2	0.0	0.0	0.0	0.1	978	MS, RI
4	*β*-myrcene	0.3	0.6	0.4	0.4	0.4	0.4	992	MS, RI
5	*α*-phellandrene	0.1	0.2	0.2	0.2	0.2	0.1	1009	MS, RI
6	limonene	0.4	0.7	0.3	0.2	0.1	0.3	1029	MS, RI
7	*β*-phellandrene	1.7	2.7	2.3	1.0	0.8	0.9	1030	MS, RI
8	eucalyptol	0.9	2.6	1.8	1.8	2.6	1.8	1032	MS, RI
9	terpinolene	0.1	0.2	0.1	0.1	0.1	0.1	1088	MS, RI
Oxygenated Monoterpenes		**3**	**5.2**	**2.6**	**3.1**	**3.4**	**3.3**		
10	linalool	0.1	0.2	0.1	0.2	0.2	0.1	1110	MS, RI
11	borneol	0.3	0.7	0.3	0.5	0.5	0.4	1176	MS, RI
12	*α*-terpineol	0.0	0.0	0.1	0.5	0.1	0.1	1205	MS, RI
13	*β*-citral	0.8	1.5	0.7	0.7	0.9	0.9	1245	MS, RI
14	*α*-citral	1.5	2.7	1.1	1.0	1.4	1.5	1277	MS, RI
15	Bornyl acetate	0.1	0.0	0.1	0.1	0.2	0.2	1284	MS, RI
16	2-undecanone	0.2	0.1	0.2	0.1	0.1	0.1	1301	MS, RI
Sesquiterpenes		**80.2**	**69.9**	**78**	**80.6**	**78.2**	**79.7**		
17	*δ*-elemene	0.1	0.0	0.1	0.1	0.2	0.0	1331	MS, RI
18	cyclosativene	0.2	0.2	0.2	0.2	0.0	0.2	1359	MS, RI
19	*α*-copaene	0.4	0.4	0.4	0.4	0.4	0.4	1369	MS, RI
20	*α*-elemene	0.7	0.6	0.7	0.7	0.8	0.7	1385	MS, RI
21	7-epi-sesquithujene	0.2	0.2	0.2	0.2	0.2	0.2	1399	MS, RI
22	*γ*-elemene	0.4	0.3	0.4	0.4	0.4	0.4	1425	MS, RI
23	*β*-farnesene	1.0	0.9	0.9	0.9	0.5	0.9	1454	MS, RI
24	germacrene	2.0	1.6	1.9	1.9	2.0	2.4	1475	MS, RI
25	*α*-curcumene	5.8	5.8	4.5	5.0	0.0	4.9	1483	MS, RI
26	*β*-selimene	0.4	0.0	0.6	0.0	0.0	0.9	1490	MS, RI
27	*γ*-muurolene	2.7	1.0	0.0	0.0	4.7	4.0	1494	MS, RI
28	**zingiberene**	**37.0**	**33.0**	**39.7**	**39.9**	**39.2**	**35.6**	**1501**	MS, RI
29	**bisabolene**	**15.1**	**12.7**	**14.1**	**16.0**	**14.9**	**14.9**	**1511**	MS, RI
30	*δ*-cadinene	0.2	0.2	0.2	0.2	0.2	0.2	1518	MS, RI
31	**sesquiphellandrene**	**14.0**	**13.0**	**14.1**	**14.7**	**14.7**	**14.0**	**1527**	MS, RI

Continued

Oxygenated Sesquiterpenes		**3.3**	**2**	**2.5**	**2.7**	**2.8**	**2.8**		
32	elemol	0.4	0.0	0.3	0.4	0.4	0.5	1552	MS, RI
33	E-nerolidol	0.6	0.5	0.5	0.5	0.6	0.5	1566	MS, RI
34	Tr-sesquisabinene hydrate	0.6	0.5	0.4	0.5	0.5	0.3	1593	MS, RI
35	zingiberenol	0.8	0.5	0.6	0.7	0.7	0.7	1616	MS, RI
36	*β*-eudesmol	0.6	0.4	0.4	0.5	0.3	0.5	1654	MS, RI
37	gérany-p-cymene	0.2	0.1	0.2	0.1	0.2	0.2	-	MS
38	(E)-1-(6.10-dimethylun-dec-5-en-2-yl)-4-methylbenzene	0.1	0.0	0.1	0.0	0.1	0.1	-	MS
Total (%)		**92.4**	**90.1**	**91.7**	**92.3**	**91.8**	**93.1**		

Table 4. Aromatic profil of *Z. zerumbet* L. from two sampling areas in Togo.

Peak N°	Component	Sampling areas and content (%)		IR/MS	Identification
		Dalanda	Kaniamboua		
Monoterpenes		**1.8**	**3**		
1	*α*-pinene	0.3	0.4	931	MS, RI
2	camphene	1.3	2.3	948	MS, RI
3	3 carene	0.2	0.3	1010	MS, RI
Oxygenated Monoterpenes		**1**	**2.4**		
4	eucalyptol	0.6	1.1	1032	MS, RI
5	camphor	0.4	1.3	1148	MS, RI
Sespuiterpenes		**27.7**	**30.9**		
6	caryophyllene	1.9	2.6	1411	MS, RI
7	**humulene**	**21.7**	**28.2**	**1450**	MS, RI
8	*α*-curcumene	0.3	_	1481	MS, RI
9	*α*-zingiberene	2.2	0.1	1493	MS, RI
10	*β*-bisabolene	0.7	_	1507	MS, RI
13	sesquiphellandrene	0.9	_	1522	MS, RI
oxygenated Sesquiterpenes		**61.9**	**50.6**		
15	caryophyllene oxyde	1.9	2.4	1575	MS, RI
16	trans sesquisabinene hydrate	4.5	5.5	1593	MS, RI
18	humulene oxyde	3.6	4.5	1604	MS, RI
25	*β*-eudesmol	0.1	0.7	1654	MS, RI
31	**zerumbone**	**51.8**	**37.5**	**1737**	MS, RI
	Total (%)	**92.4**	**86.9**		

Among Monoterpernes, camphene content among the samples varied from 1.8% (Badou) to 4.6% (Kaniamboua); *β*-phellandrene (0.8% - 2.7%) was detected in low amount and *α*-pinene (0.4% - 1.2%) in trace amount. Other oxygenated compounds identified in trace amount in the samples wers *α*-citral (1.0% - 2.7%) and *β*-citral (0.7% - 1.5%).

Despite the variability in the composition of *Z. officinale* essential oils from different sampling areas, a similar trend was observed in the composition of the major elements. Thus zingiberene (33 % - 39%), Basibolene (12.7 - 16%) and sesquiphellandrene (13% - 14.7%) are mainly present in the essential oils of all *Z. officinale* samples. However these results are slightly different with those reported elsewhere. Zingiberene, sesquiphellandrene and farnesene were found to be major components of *Z. officinale* from India and Nigeria respectively [10] [30], Zingiberene, ar-curcumene, *β*-sesquiphellandrene, and *β*-bisabolene were identified as major components in Australian *Z. officinale* [43] while zingiberene, (E.E)-*α*-farnesene and geranial were reported as major components in Malaysian *Z. officinale* [44].

Z. Zerumbet essential oil from the two sampling areas Dalanda and Kaniamboua showed high predominance of oxygenated sesquiterpenes (61.9% and 50.6%) and sesquiterpenes (27.7% and 30.9%) and a low predominance of monterpenes (1.8% and 3%) and oxygenated monoterpenes (1% and 2.4%) respectively in Dalanda and Kaniamboua. Thirty-one compounds were identified accounting for 92.4% and 86.9% respectively for Dalanda and Kaniamboua samples.

Zerumbone was the major oxygenated Sesquiterpenes observed in all the samples and the content varied from 37.5% (Dalanda) to 51.8% (Kaniamboua). The other oxygenated Sesquiterpenes found in notable amount were trans sesquisabinene hydrate from 4.5% (Dalanda) to 5.5% (Kaniamboua), humulene oxyde from 3.6% (Dalanda) to 4.5% (Kaniamboua), caryophyllene oxyde from 1.9% (Dalanda) to 2.4% (Kaniamboua). The *β*-eudesmol was found in trace amount (0.1% - 0.7%).

The second highest component identified was humulene, a sesquiterpene that was found in amount varying from 21.7% (Dalanda) to 28.2% (Kaniamboua). Caryophyllene (1.9% - 2.6%) and *α*-zingiberene (0.1% - 2.2%) were found in notable amount while *β*-bisabolene (not detected to 0.7%) and sesquiphellandrene (not detected to 0.9%) was found in trace amounts.

The major components found in *Z. zerumbet* samples (zerumbone and *α*-humulene) are different from those reported elsewhere probably due to geographic disparities. (z)-citral, camphene, sabinene and zingiberene were reported as major component in *Z. zerumbet* sample from Vietnam [20] while zerumbone, *α*-caryophyllene and camphene were found to be main components in samples from Bangladesh [19] and North-East India [45]. However, our findings are similar with those reported in Malaysia [46], in India [47], and in Reunion Island [37].

4. Conclusion

On the basis of this study, it may be concluded that *Z. zerumbet* and *Z. officinale*, growing widely in Togo, can be utilized as the sources of isolation of natural zerumbone and humulene; and zingiberene, basibolene and sesquiphellandrene respectively. Samples from Danyi-Apéyémé (39.7%), Badou (39.9%) and Blitta-Tadjan (39.2%) have comparatively high content of zingiberene which is the major component of *Z. officinale* while sample from Dalanda (51.8%) has high content of zerumbone which is the major component of *Z. zerumbet*.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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