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Chemical Characterization of Priprioca (*Cyperus articulatus)* Leaf Essential Oil from Brazil Amazon Basin

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Abstract

The *Cyperus articulatus* is a tall plant that grows mainly near the edges of rivers. One of its common names is priprioca. In this work was used priprioca native to the Amazon basin, where native tribes have used it as a medicine for hundreds of years. The essential oils from the rhizomes of *Cyperus articulatus* collected in Brazil were extracted by hydrodistillation and analyzed by two-dimensional chromatography with the objective of attaining very high-resolution second dimension separations. Recent studies on the chemical composition of essential oil by conventional have shown that this grass contains an amount of almost 50 constituents. The compound includes flavonoids, polyphenols, saponins, tannins, and terpenes. The main constituents of essential oil from Brazil were *α*-cyperone and cyperotundone. These latter two compounds are believed to be effective pain relievers, working in the same manner as aspirin and ibuprofen, and may also possess antimalarial properties. In this paper, the chemical composition of the essential oil of *Cyperus articulatus* was performed by Comprehensive Two-Dimensional Gas Chromatography coupled with Quadrupole Mass Spectrometric detection. This technique allowed the identification of about 100 constituents of the oil of *Cyperus articulatus*. Obviously, two-dimensional chromatography was better when compared to conventional chromatography.

Keywords

Cyperus Articulatus, Priprioca, Chromatography, Composition Essential Oil

1. Introduction

Cyperus articulatus, (Family Cyperaceae, genus Cyperus, species Articulatus) is also well known in Brazil as Priprioca. The Cyperaceae plant family which include

approximately 36 genera and about 128 species of Cyperus. Although native to the Amazon, Priprioca can be found in many other tropical areas and countries, including the southern United States, Africa, Asia, Australia, and across the South American continent. It can be found growing along side the Nile River in Africa just as it grows alongside the Amazon River in South America [1].

Priprioca is a type of reed-like tropical grass called a "sedge-grass." It grows in small clusters and routinely reaches over 6 feet (2 meters) in height and their stems are fibrous, cylindrical and hollow. The stem narrows as it grows upward turning into spiked blades of shiny grass, which range in color from bright yellow-green to dark forest green, and can project a purplish inflorescence under the right lighting conditions. During the summer season, the grass produces many tiny white flowers at the top of the stalk, which has been described as being similar to the tiny white flowers produced by wheat grass [2].

Priprioca contains flavonoids, polyphenols, saponins, tannins, terpenes and sugars. Many of its biological actions are attributed to various sesquiterpenes called cyperones which are also found in other Cyperus plants in the family. Two of these chemicals, called cyperotundone and cyperone were found in Brazilian species as the main compounds of the oil [3]. The major volatile compound found on essential oil is, ranging from to 2.3% although percentages of up to 26.15% have already been reported [4].

There is discussion on the literature regarding its chemical composition [5]. The terpene chemicals documented in Priprioca thus far include: cyperone, pinene, carophyllene oxide, cyperotundone, patchoullenone and mustakone.

Literature has a relatively high number of studies showing the chemical composition of the complex matrices such as priprioca essential oil. The authors found about 50 chemical compounds by gas chromatography (GC) employed as universal analytical technique [6].

A comprehensive two-dimensional gas chromatography (GC × GC) has a high capacity of separation and it is a promissing alternative for complex mixture [7]. There are several reports in the literature concerning the application of GC × GC to the analysis of fragrances, aromas and essential oils [8], but neither of them include *Cyperus articulatus* essential oil from Brazil. In this work were found about 100 chemical compounds by GC × GC-qMS. As for the detection, quadrupole mass spectrometry (qMS) has been pointed as a viable alternative to time-of-flight mass spectrometry (ToF-MS). The former has much lower cost and ultimate generation rapid-scanning qMS instruments are quite suitable for GC×GC instrumental analysis requirements [9].

In this work the objective is to compare results by GC×GC-qMS with those previously reported in the literature on the essential oil compositions of *C. articulatus* from Brazil by GC-MS. The components of the oil must be identified base on the comparison of their retention indices and mass spectra with those standards, NIST library mass spectra data based of the GC/MS system and published data.

2. Experimental

2.1. Raw Material

Rhizomes of *Cyperus Articulatus* were collected in Amazon State—Brazil. This brazilian plant was identified by one of authors of this paper, P. T. B. Sampaio. Raw material was distilled for 6 hours in an industrial 1500 L iron reactor to extract the essential oils. The final product was separated from water after reaching room temperature and kept at a temperature of −10˚C for further analysis.

2.2. Analysis of Essential Oil

The chemical analyses were performed on a GCMS-QP2010 Plus gas chromatograph from Shimadzu, adapted to work as GC × GC-qMS. The technology called quadrupole mass spectrometry (qMS) was used for the detection on GC × GC instrumental analysis. Data were acquired using the GC solution software (Shimadzu). Dimensional chromatograms were generated by using the GC Image software (Shimadzu).

The column combination employed consisted of a polar 30 m × 0.25 mm × 0.25 μm, length of 98 m, HP-5 (5% phenyl-dimethylpolysiloxane), fused-silica column connected to a detector-linked (the modulating system was applied every 6 s) high-resolution 1.0 m × 0.1 mm × 0.1 μm DB-Wax (Polyethyleneglycol-PEG) apolar analytical column.

The conventional GC and GC × GC application was operated as follows: column oven temperature of 60˚C; injection temperature of 250˚C; injection mode in the split-flow; carrier gas: H_2; total flow: 93.6 mL/min; column flow: 0.60 mL/min; linear velocity: 23.3 cm/s; purge flow: 3.0 mL/min, split ratio to C_{15} region and C_{10} region, respectively: 150:1 and 50:1.

The total program time was 60 min.

The qMS was operated as follows: H2 flow: 93.6 mL/min; air flow: 400.0 mL/min; make up (He): 50.0 mL/min; sampling frequency: 125 Hz and 250 Hz in the split-flow. The chromatograph was setting in temperature programmed as follows: 60˚ until 210˚C at 3˚C/min. The carrier gas he was used at a flow of 0.6 mL/min. The injection port was set at 250˚C. Samples were injected using a split ratio of 1:100. MS operating parameters: transfer line temperature: 240˚C; electron impact ionization at 70 eV with mass scan range of 40 - 284 *m/z* at a sampling rate of 0.03 scan/s; ion source temperature: 200˚C. Compounds were identified by computer search using digital libraries of mass spectral data [10] and by comparison of authentic mass spectra [11] and their retention indices, relative to C_8-C_{20} n-alkane series in a linear temperature-programmed run. GC × GC-qMS and GC-qMS analyses were performed using the same gas chromatograph and MS operating conditions and temperature program, described above. Data were acquired by GCMS Real Time Analysis (GCMS Solutions, Shimadzu Corp.) and processed using GC Image software, ver.2.1 (GC Image, LLC, Lincoln, NE). Proper software for GC × GC data manipulation (GC Image 2.0, Zoex Corp.—Houston, TX) was used for data handling. A value of spectral similarity above 900 was fixed as an

acceptable Identity Spectrum Match factor resulting from the NIST Identity Spectrum Search algorithm (NIST MS Search 2.0).

3. Results and Discussion

In order to compare one-dimensional (1D) reference LTPRI values (commercial Libraries) with experimental LTPRI obtained in this work, the sample was spiked with a solution of n-alkanes. The retention indexes were calculated by GCMS Solution software for the compounds, using the van den Dool and Kratz formula [12]. The compounds were tentatively identified with a combination of the mass spectral similarity and the LPTRI. A previous work reports the use of one-dimensional retention indexes to GC × GC data [13].

GC × GC-qMS chromatographics runs identified x compounds in the essential oil extracted (**Table 1** contains the identified compounds by GC-qMS—all without bold). **Table 1**, their respective experimental linear retention indexes and literature LTPRI values (from Adams [14] and NIST [15]), for the samples analyzed under similar conditions.

Table 1. Identified compounds and the respective literature and calculated retention indexes obtained by GC-qMS (LTPRI Calc I, LTPRI Lit II and % Peak area).

Compound	% Average spectral similarity		
	LTPRI Calc I	LTPRI Lit II	% Peak area
Pinene <*α*-> (97)	935	932	0.8
Camphene (95)	952	946	0.02
Thuja-2,4-(10)-diene (97)	954	953	0.01
Verbenene (97)	962	960	0.30
Sabinene (95)	975	969	0.4
Pinene <*β*-> (95)	979	974	0.7
Cymene <p-> (96)	1024	1020	0.2
Limonene (96)	1029	1024	0.31
Cineole <1, 8-> (95)	1031	1029	0.01
Cymenene <p-> (97)	1088	1089	0.01
Campholenal <*α*-> (97)	1125	1122	0.4
Pinocarveol<trans-> (95)	1137	1135	0.3
Verbenol <trans-> (95)	1143	1140	0.15
Pinocarvone (97)	1165	1160	0.73
Mentha-1,5-dien-8-ol <p-> (95)	1169	1166	0.01
Terpineol (96)	1176	1174	0.2
Cymen-8-ol <p->	1184	1176	0.01
Myrtenal + Myrtenol (97)	1194	1195	0.4
Verbenone (97)	1205	1200	0.01

Continued

Carveol <trans-> (90)	1216	1215	0.45
Carvone (97)	1241	1239	0.5
Cymen-7-ol <p-> (97)	1286	1289	0.5
Cypera-2,4-diene	1359	*	1.74
Copaene <α-> (97)	1373	1374	1.58
Elemene <β-> (91)	1391	1389	0.32
Cyperene (98)	1395	1398	13.15
Caryophyllene <β-> (99)	1419	1417	1.97
Guaiene <α-> (98)	1440	1437	0.20
Humulene <α-> (95)	1455	1452	1.15
Rotundene (97)	1458	1457	0.4
Germacredene D (97)	1486	1484	0.7
Eudesma-2,4,11-triene	1468	*	0.01
Selinene <β->	1489	*	15.79
Selinene <α-> (95)	1496	1498	0.9
Bulnesene <α-> (96)	1507	1509	0.8
Cadinene <δ-> (95)	1523	1522	0.7
Calamenene <trans->	1528	*	0.3
Calacorene <α-> (96)	1543	1544	1.3
Ledol (95)	1566	1602	0.6
Caryophyllene oxide (97)	1581	1582	2.3
Humulene epoxide II (96)	1604	1608	0.92
Copaen-4-α-ol <β-> (97)	1589	1590	0.45
Dill apiole (97)	1621	1620	0.9
Patchoulenone	1615	*	1.1
Caryophylla-4(14)-8(15)-dien-5α-ol	1642	*	0.9
M218	1644	*	1.2
Eudesma-3,11-dien-5-ol	1632	*	0.01
Mustakone	1677	*	1.3
Cyperotundone	1693	*	16.5
M220	1741	*	1.1
Cyperone <α->	1754	*	23.6
Aristolone (96)	1763	1762	1.5

Nyasse *et al.* [16], studied the composition of priprioca essential oil from rizhomes by conventional gas chromatography. A comparison of the essential oil analysis obtained in this work shows that the compositions are similar. Almost all compounds were found in both works and the major compounds are the same.

Figure 1 and Figure 2 present the chromatograms obtained by GC × GC-qMS. Using GC × GC-qMS, it was possible to identify a much larger number of compounds (about 2.5 times more) when compared with study by Mondello *et al.* [17].

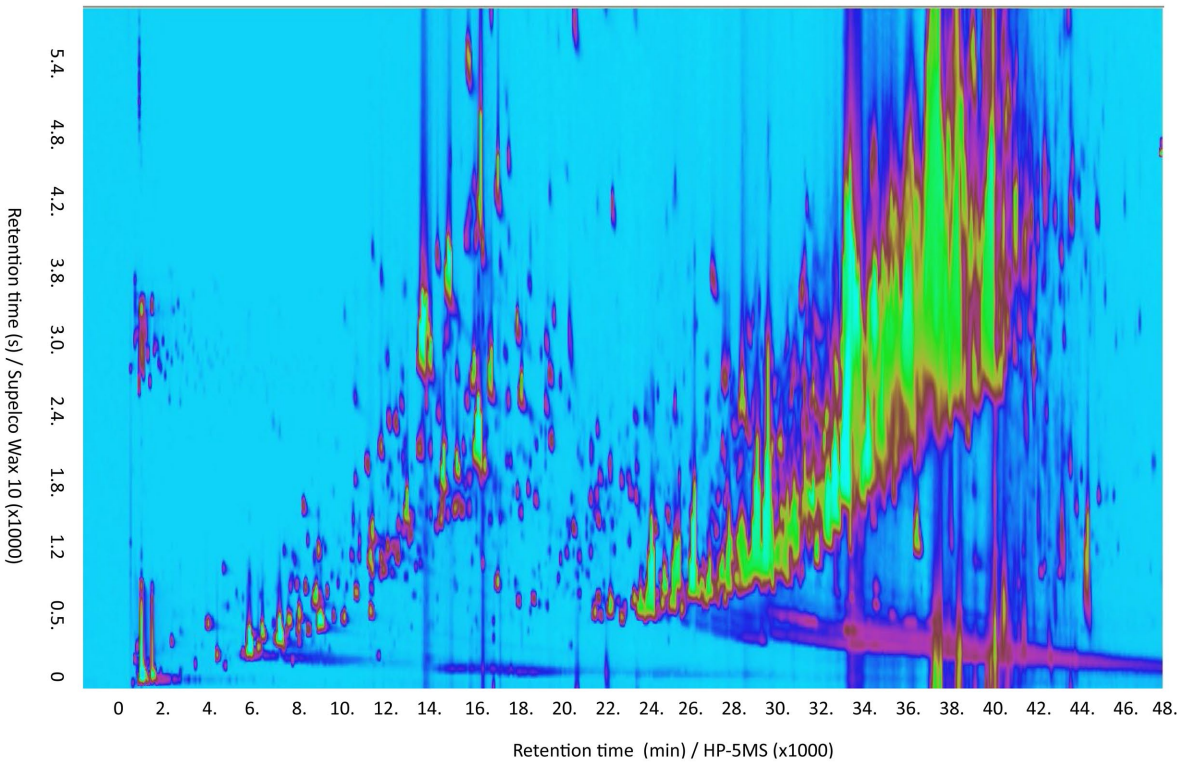

Figure 1. GC × GC-qMS chromatogram of priprioca essential oil sample extracted from rizhomes $t_{R\,1}$: first dimension retention time. $t_{R\,2}$: second dimension retention time. The split ratio used was 1:50 for analysis of C_{10} region.

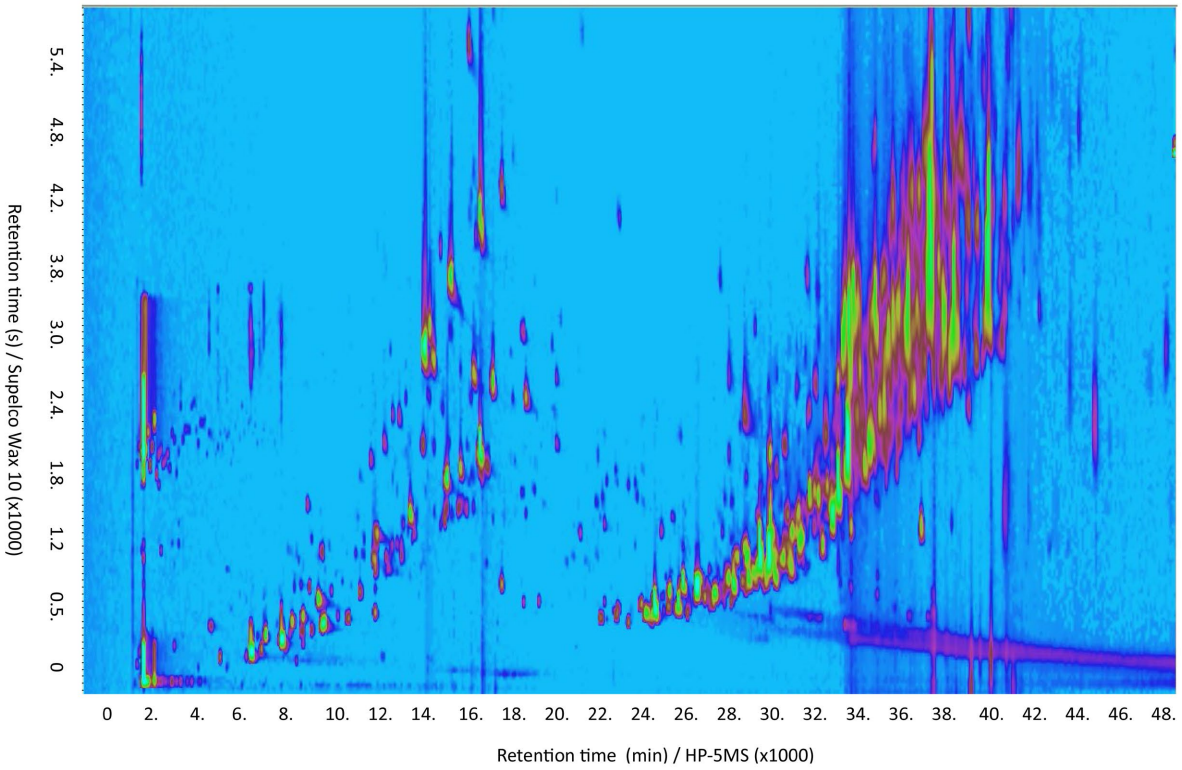

Figure 2. GC × GC-qMS chromatogram of priprioca essential oil sample extracted from rizhomes $^{1}t_{R}$: first dimension retention time. $^{2}t_{R}$: second dimension retention time. The split ratio used was 1:150 for analysis of C_{15} region.

Two different split were resolved as a result of the higher separation capacity and mass spectral quality. Figure 1 reveals the real complexity of the sample for C_{10} region. For analysis of C_{10} region, peaks became narrower and more intense when GC × GC runs occurred under split 1:50. Figure 2 reveals the real complexity of the sample for C_{15} region. For analysis of C_{15} region, GC × GC runs occurred under split 1:150.

These two split ratio are strategy used to increase MS sensitivity on different regions of compounds such as C_{10} and C_{15}. It was possible to compare the peak area percent of the all compounds by GC × GC-qMS on both chromatograms.

The use of GC × GC-qMS enabled good improvement in separation and number of identified peaks of priprioca essential oil. All compounds identified by GC-MS were found in the sample by GC × GC-qMS. More quantity of compounds were identified by GC × GC-qMS analyses. The results obtained show that essential oil extracted have a more complex composition than that obtained by conventional gas chromatography. Table 1 and Table 2 show the identified components by GC-MS and GC × GC-qMS, respectively.

Table 2. Identified compounds and the respective literature and calculated retention indexes obtained by GC-qMS (tR1, tR2, LTPRI Calc a and LTPRI Calc b).

Compound	% Average Spectral Similarity			
	tR 1/s min	tR2/s min	LTPRI Calc a	LTPRI Calc b
Pinene	7.70	0.81	938	932
Camphene	7.80	0.54	941	946
Thuja-2,4-(10)-diene	8.10	0.45	951	953
Pinen-3-ol <*cis*->	8.30	0.37	957	*
Verbenene	8.40	0.46	960	961
Sabinene	8.60	0.53	967	969
Pinane <trans->	8.80	0.64	973	969
Pinene	8.90	0.69	974	974
Pinane <*cis*->	9.00	0.82	980	982
Myrcene	9.20	0.63	986	988
Pinen-10-ol <2->	9.70	1.18	1002	*
Terpinene	10.10	0.75	1012	1014
Cymene <p->	10.40	0.87	1019	1020
Cymene <o->	10.50	0.74	1022	1019
Limonene	10.70	0.81	1027	1024
Cineole <1,8->	10.80	1.16	1029	1026
Terpineol	10.90	1.23	1032	*
Ocimene < *cis*>	11.00	1.31	1034	1032
Ocimene <*trans*>	11.20	0.85	1039	1044
Octadiene, 3,7-dimethyl-, <1,6	11.30	0.57	1042	*
Thujaketone	11.40	0.49	1042	*
Cymenene <p->	13.10	1.82	1088	1089

Continued

Camphenone <6->	13.30	1.23	1092	1095
Camphenol <6->	13.40	0.36	1095	1111
Pinene hydrate <*trans*->	14.10	1.15	1120	1119
Campholenal	14.60	2.39	1123	1122
Limonene oxide <*cis*->	15.00	2.45	1132	1132
Pinocarveol <*trans*->	15.10	1.96	1134	1135
Verbenol <*cis*->	15.30	1.14	1138	1137
Verbenol <*trans*->	15.50	1.07	1139	1140
Pinocarvone	16.50	3.61	1166	1160
Thujanol<3->	16.60	2.67	1168	1164
Mentha-1,5-dien-8-ol <p->	16.70	1.86	1171	1166
Pinocamphone <*cis*->	16.80	2.09	1173	1172
Terpinen-4-ol	17.20	1.52	1182	1174
Cymen-8-ol <p->	17.30	1.83	1183	1176
Mentha-1(7),2-dien-8-ol <*trans*- p->	17.40	1.57	1184	1187
Myrtenal + Myrtenol	17.70	2.38	1193	1195
Verbenone ou Pinenone	18.50	0.85	1210	1204
Carveol <*trans*->	18.70	4.22	1216	1215
Carveol <*cis*->	19.10	0.94	1225	1226
Carvone	19.80	2.41	1240	1239
Cymen-7-ol <p->	21.80	2.10	1284	1289
Longipinene	24.40	0.75	1343	1350
Cypera-diene2,4	25.00	0.59	1360	*
Copaene	26.10	1.13	1381	1374
Cyperene	26.50	1.34	1396	*
Elemene	26.80	0.72	1397	1389
Gurjunene	27.30	1.45	1409	1409
Caryophyllene	27.40	0.98	1412	1408
Caryophyllene < *trans*>	28.00	1.36	1426	1417
Gurjunene	28.10	1.87	1428	1431
Longipinene epoxide	28.60	1.85	1440	*
Guaiene	29.00	1.13	1444	1437
Humulene	29.30	3.62	1456	1452
Rotundene	29.60	1.66	1463	1457
santalene	29.70	2.09	1466	1457
Gurjunene	30.10	2.18	1475	1475
Murolene	30.20	2.25	1478	1478
Germacredene D	30.60	2.39	1485	1484
Selinene	30.70	2.73	1490	1489
Guaiene <cis>	30.80	2.84	1492	1492
Cadina-1,4-diene	30.90	3.04	1494	1495

Continued

Selinene	31.00	2.62	1497	1498
Guaiene <trans>	31.30	3.05	1504	1502
Eudesma,2,4, 11-triene	31.40	3.29	1505	*
Guaia-1(10), 11-diene	31.50	2.71	1507	*
Bulnesene	31.70	2.43	1509	1509
Germacrene A	31.90	3.03	1519	1508
Dodecadienol	32.00	1.82	1522	*
Bisabolene epoxide <*trans*- Z-	32.10	1.94	1524	*
Cadinene	32.30	1.85	1529	1522
Calamenene <*trans*->	32.50	1.46	1534	1521
Calacorene	33.20	1.77	1549	1544
Germacrene B	33.30	1.63	1552	1559
Nerolidol	33.60	1.65	1561	*
Calacorene	33.70	1.79	1564	1564
Calamenene	33.90	1.71	1569	*
Ledane	34.50	2.14	1584	*
Ledol	34.70	1.48	1589	1602
Caryophyllene oxide	34.90	2.66	1594	1582
Humulene epoxide II	35.30	2.72	1603	1608
Copaen-4-ol	35.40	1.97	1605	1590
Eudesm-4(14)-en-11-ol	35.50	1.83	1607	*
Ledene oxide II	35.60	3.33	1609	*
Dill apiole	35.70	2.80	1612	1620
Ledane	35.80	2.45	1614	*
Patchoulenone	35.90	3.59	1617	*
Eudesma-3,11-dien-5-ol	36.10	2.70	1624	1639
Caryophylla – 4(14), 8(15) –dien-5-ol	36.80	2.82	1641	*
Eudesmol	36.90	3.25	1643	1652
Longipinene epoxide	37.20	3.23	1653	*
Mustakone	38.30	1.44	1680	1676
Bisabolene oxide	38.40	4.46	1682	1696
Germacra-4(15),(14)trien-4-ol	38.50	4.19	1685	*
Guaia-5,11-diene	38.70	4.10	1690	*
Cyperotundone	38.80	3.67	1694	1695
M218	39.20	4.12	1703	*
Cyclolongifolene oxide	39.50	4.25	1711	*
Bisabolene <cis>	39.70	4.28	1717	*
Solavetivone	39.90	4.15	1722	*
Bisabolene epoxide <trans-Z-*α*	40.00	4.53	1726	*
Bisabolene epoxide <cis-Z-*α*>	40.10	4.76	1728	*
Cyperone	41.00	3.84	1754	*
Aristolone	41.40	4.88	1763	1762

Some of the more commonly encountered monoterpenoid hydrocarbons can be formed by dehydration of alcohols and so their presence in essential oils could be as artifacts arising from the extraction process. As sesquiterpenoids contain 15 carbon atoms they have lower volatilities and hence higher boiling points than monoterpenoids. Therefore, fewer of them (in percentage terms) contribute to the odor of essential oils but those that do often have low-odor thresholds and contribute significantly as end notes [18].

As can be seen by **Table 1** (the numbers in the parenthesis are the % peak area and calculated retention index, respectively), some compounds were identified by conventional gas chromatography. The compounds pinen-3-ol <*cis*->, pinane <*trans*->, pinane <*cis*->, myrcene, pinen-10-ol <2->, terpinene, cymene <o->, terpineol, ocymene <*cis*>, ocymene <*trans*>, octadiene, 3, 7-dimethyl <1,6>, thujaketone, camphenone <6->, camphenol <6->, pinene hydrate <*trans*->, limonene oxide <*cis*->, verbenol <*cis*->, thujanol <3->, pinocamphone <*cis*->, terpinen-4-ol, mentha-1(7)-2-dien-8-ol <*trans*-p->, carveol <*cis*->, naphtalenedione <2-methoxy-8-methyl-1,4->, ylangene <oxo>, 2(3H)–naphtalenone <4,4,a, 5,6,7,8-hexahydro-4,5-dimethyl,3, longipinocarvone, gurjunene, longipinene epoxide, santalene, murolene, guaiene <*cis*>, cadina-1,4-diene, guaiene <*trans*>, guaia-1(10),11-diene, germacrene A, dodecadiene, bisabolene epoxide, germacrene B, nerolidol, calacorene, ledane, eudesm-4(14)-en-11-ol, eudesmol, cyclolongifolene oxide, germacra-4(15),5,10–(14)-trien-4α-ol, guaia-5,11-diene, longipinene epoxide, bisabolene oxide, bisabolene <*cis*->, solavetivone, bisabolene epoxide were identified only by GC × GC-qMS in sample (**Table 2**). The library can mistake some components like isomers, but it is also possible that co-elutions results in difficult in the identification process. This can be the case of Cadina-1,4-diene and amorphene, whose retention indexes are close. This is a consequence of the high number of sample components and the relative low separation capacity of conventional gas chromatography. Major compounds as show similar % peak area in the essential oil.

GC × GC-qMS analysis showed fewer differences in the identification, mainly when one consider the higher number of identified components. This shows us that GC × GC-qMS can be more precise and hence more reliable to the chemical characterization of samples like essential oil obtained from rizhomes of *C. articulatus*.

4. Conclusion

This project showed that compositions of the analyzed samples are very similar when one considers the complexity of essential oils. This could be concluded only by comprehensive two-dimensional gas chromatography coupled with quadrupolar mass spectroscopy, because the separation capacity of conventional gas chromatography is quite limited in the case of complex samples. Differences in the minor compounds content among the essential oils analyzed can be corrected relatively ease when one wishes to reach the better fragrance quality. The economical

interest in this raw material increases the importance of further investment in this research.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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