Chemical composition of the essential oil of Myrcia loranthifolia (Myrtaceae) leaves grown in Atlantic Forest and Dry Forest of Brazil

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Abstract

The present study evaluated the chemical composition of the leaf essential oil of *Myrcia loranthifolia* from Atlantic Forest and Dry Forest of Brazil. The oil showed a mean yield of 0.23–0.33%. From 26 to 32 compounds were identified by GC–MS depending on the geographical origin of the plant. (*E*)–Caryophyllene (47.80%) and germacrene D (10.07%) predominated in the oil of samples from the Atlantic Forest. In Dry Forest, the main constituent identified in the oil was also (*E*)–caryophyllene, but it was detected in a lower concentration (15.59%). *Cis*–calamenene (11.40%), the second major constituent found in the oil of plants from Dry Forest, was not identified in samples from the Atlantic Forest. The qualitative and quantitative differences found may be due to local abiotic factors or inherent to genetic characteristics of the plants. The essential oil of *M. loranthifolia* constitutes one of the largest sources of (*E*)–caryophyllene yet unexplored.

1 Introduction

The family Myrtaceae is known for its diversity of species, estimated at almost 6,000, distributed in 132 genera (Christenhusz and Byng 2016). Twenty-nine genera and 1193 species of the family are found in Brazil (The Brazil Flora Group – BFG 2015). One of the striking characteristics of this family is the presence of essential oil secretory structures in vegetative and reproductive organs. Some representatives of this family have fruits that are edible or used as spices, and others have ornamental and medicinal potential. The species are particularly rich in essential oils (de Paulo Farias et al. 2020; Maiolini et al. 2022).

Among Myrtaceae genera, *Myrcia* DC., a heterotypic synonym of *Calyptranthes* Sw., has been described as occurring in different phytogeographic domains, including Brazilian Atlantic Forest and Brazilian Dry Forest (Santos et al. 2023). The chemical composition of the essential oil of some species of the genus *Myrcia* has also been described (Henriques et al. 1997; Pereira et al. 2010; Dos Santos et al. 2014; Alves et al. 2016; Rosa et al. 2016; Tietbohl et al. 2020; de Moraes et al. 2022; Maiolini et al. 2022). However, to our knowledge, the chemical profile of the leaf essential oil of *Myrcia loranthifolia* (DC.) G.P.Burton & E.Lucas [synonym of *Calyptranthes dardanoi*], a species registered from the Brazilian Atlantic Forest (BAF) and Brazilian Dry Forest (BDF), and its bioactive potentialities are still unknown.

Due to the occurrence of *M. loranthifolia* in different phytogeographic domains in Brazil, and consequently distinct climates, the essential oil content and composition of this species were investigated for first time. We hypothesize that the oil content and composition will be influenced by the geographical origin of the plant, with implications of their possible biological activity.

2 Materials and methods

Plant material – *Myrcia loranthifolia* was collected at Vale do Catimbau National Park, Buíque (BDF) (8°35’05.6” S, 37°14’34.1” W) and Usina São José, Igarassu (BAF) (7°49’55.8” S, 35°00’21.2” W), both in

Pernambuco state, northeastern Brazil. The species was identified and deposited in the Dárdano de Andrade Lima Herbarium – IPA under voucher numbers 92921 and 93554, respectively.

Essential oil extraction – After collection, the leaves were dried at room temperature and then crushed. The oil was extracted by hydrodistillation with a Clevenger apparatus. A ratio of 100 g of leaves to 500 mL of distilled water was used, for 4 hours (Pereira et al. 2011). The extracted oil was stored in amber flasks at -15°C temperature. The essential oil yield was expressed in percentage (%) from the volume (mL) of oil obtained per dry mass (g) of leaves (Santos et al. 2014).

Analysis of the essential oils – For chemical analysis, 1 mg of the essential oil was diluted in 1 mL of bidistilled hexane and 1 µL of the solution was injected in the split mode at 1:50 ratio into a gas chromatograph coupled to an Agilent 5975C series GC–MSD mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) equipped with an Agilent J&W HP–5 column (60 m × 0.25 mm x 0.25 µm). The GC–MSD conditions and the identification of the oil components were according to the previously reported method (da Silva Barbosa et al. 2020). The compounds identified by GC–MSD were quantified by gas chromatography and flame ionization detection (GC–FID, Thermo Trace GC, Thermo Scientific, Waltham, MA, USA). A VB-5 apolar column (60 m × 0.25 mm x 0.25 µm, ValcoBond®, Valco Instruments Company Inc., Houston, TX, USA) was used. Programming of the GC–FID followed the methodology described above.

Statistical analysis – The results of essential oil yields and composition were expressed as mean ± standard deviation and analyzed using GraphPad Prism 7.0 software (GraphPad Software, Inc., San Diego, CA).

3 Results

The essential oil obtained from *M. loranthifolia* leaves collected in BAF and BDF showed a mean yield of 0.23% and 0.33%, respectively. The oil was clear and colorless, with a strong odor. It was not possible to differentiate the oils from BAF and BDF by these sensory characteristics.

We identified 26 compounds in the essential oil of individuals from BAF and 32 in those from BDF (Table 1), totaling 96.58% and 90.45% of the identified compounds, respectively. In the samples analyzed, there was a predominance of sesquiterpene hydrocarbons (90.17% and 79.10% in plants from BAF and BDF, respectively). Compounds of phenolic nature were minority in the oil of *M. loranthifolia* (< 1%).

Table 1
Chemical composition of the essential oil of *Myrcia loranthifolia* leaves occurring in Brazilian Atlantic Forest (BAF) and Brazilian Dry Forest (BDF)

	Chemical composition				
	M. loranthifolia (BAF)		*M. loranthifolia* (BDF)		Literature
Compound	RI [a]	%	RI [a]	%	RI [b]
α-pinene	932	0.06 ± 0.01	932	0.03 ± 0.01	930
β-pinene	974	0.12 ± 0.02	974	0.07 ± 0.02	973
1,8-cineole	1026	0.23 ± 0.01	1026	0.14 ± 0.01	1029
fenchone	1083	0.09 ± 0.01	1083	0.07 ± 0.01	1086
nonanal	-	-	1100	0.02 ± 0.01	1104
δ-elemene	1335	0.14 ± 0.01	1335	0.76 ± 0.01	1337
α-cubebene	1348	1.00 ± 0.02	1348	1.51 ± 0.03	1349
α-copaene	1376	2.71 ± 0.04	1376	2.83 ± 0.04	1376
β-bourbonene	1387	2.21 ± 0.03	1387	1.45 ± 0.03	1385
β-elemene	1389	5.29 ± 0.04	1389	4.56 ± 0.18	1392
methyl eugenol	-	-	1403	0.94 ± 0.01	1400
α-gurjunene	1409	0.14 ± 0.02	1409	0.75 ± 0.01	1409
(*E*)-caryophyllene	1417	**47.80** ± 0.15	1417	**15.59** ± 0.11	1420
β-copaene	1430	0.82 ± 0.03	1430	1.54 ± 0.04	1429
aromadendrene	1439	0.19 ± 0.01	1439	1.11 ± 0.03	1439
α-humulene	1452	**9.09** ± 0.05	1452	**9.86** ± 0.10	1454
allo-aromadendrene	1458	0.72 ± 0.02	1458	2.02 ± 0.05	1461
germacrene D	1480	**10.07** ± 0.04	1484	**10.22** ± 0.13	1482
bicyclogermacrene	1500	3.99 ± 0.02	1500	**9.62** ± 0.15	1497
germacrene A	1508	1.15 ± 0.02	1508	2.81 ± 0.03	1506
γ-cadinene	1513	0.58 ± 0.03	-	-	-

[a] Retention Index (RI) calculated by the retention time relative to that of a series of C9-C30 *n*-alkanes on HP-5 capillary column. [b] Values obtained from Adams (2009). % = area of the compound in relation to the total area of the chromatogram. Bold numbers represent major constituents of the oil. Values are mean of three determinations ± standard deviation. - = compound not detected (concentration < 0.01%)

Chemical composition					
cis-calamenene	-	-	1528	**11.40** ± 0.11	1524
δ-cadinene	1522	3.80 ± 0.04	-	-	-
trans-cadina-1,4-diene	1533	0.25 ± 0.02	1533	2.47 ± 0.03	1533
α-cadinene	1537	0.15 ± 0.01	-	-	-
α-calacorene	1544	0.07 ± 0.01	-	-	-
germacrene B	-	-	1559	0.60 ± 0.02	1558
palustrol	-	-	1567	0.22 ± 0.03	1568
spathulenol	-	-	1577	1.99 ± 0.03	1578
caryophyllene oxide	1582	2.78 ± 0.03	-	-	-
hexadecane	-	-	1600	1.60 ± 0.02	1597
ledol	-	-	1602	0.59 ± 0.01	1604
humulene epoxide II	-	-	1608	0.56 ± 0.02	1610
α-muurolol	1644	1.74 ± 0.03	-	-	-
epi-*α*-muurolol	-	-	1640	1.40 ± 0.02	1643
α-cadinol	1622	1.39 ± 0.02	1652	0.95 ± 0.03	1655
heptadecane	-	-	1700	2.54 ± 0.04	1698
octadecane	-	-	1800	0.23 ± 0.01	1797
Total		96.58%		90.45%	
Monoterpene hydrocarbons		0.18		0.10	
Oxygenated monoterpenes		0.32		0.21	
Sesquiterpene hydrocarbons		90.17		79.10	
Oxygenated sesquiterpenes		5.91		5.71	
Phenylpropanoids		-		0.94	
Others		3.42		9.55	

[a] Retention Index (RI) calculated by the retention time relative to that of a series of C9-C30 *n*-alkanes on HP-5 capillary column. [b] Values obtained from Adams (2009). % = area of the compound in relation to the total area of the chromatogram. Bold numbers represent major constituents of the oil. Values are mean of three determinations ± standard deviation. - = compound not detected (concentration < 0.01%)

According to Table 1, the major component of the essential oil of *M. loranthifolia* from BAF was (*E*)–caryophyllene (47.80%), followed by germacrene D (10.07%) and *α*-humulene (9.09%). In plants from BDF, the major components identified were: (*E*)–caryophyllene (15.59%), *cis*-calamenene (11.40%), germacrene D (10.22%), *α*-humulene (9.86%), and bicyclogermacrene (9.62%). Total ion chromatograms of the essential oil from *M. loranthifolia* leaves occurring in BAF and BDF are shown in Fig. 1 and Fig. 2, respectively.

4 Discussion

Species of the genus *Myrcia* have already been investigated for volatile oil content and composition. The oil content has been recorded to vary from 0.05–1.70% (Henriques et al. 1997; Zoghbi et al. 2003; Stefanello et al. 2007; Raposo et al. 2018). Our present findings for *M. loranthifolia* from BAF and BDF are within this interval, which suggested that the yields of essential oils from different Myrtaceae species vary according to the plant species and collection environment.

In most studies, *Myrcia* species were collected in forest areas (e.g., Amazon and Atlantic Forest), Cerrado (Savanna), Restinga (Coastal sandy) and Caatinga (Dry Forest), which have geoclimatic characteristics distinct (Dos Santos et al. 2014; Tietbohl et al. 2020; de Moraes et al. 2022; Maiolini et al. 2022). The latter is an ecosystem, for example, is characterized by a semi-arid climate with irregular rainfall distribution and extended droughts (de Queiroz et al. 2017). The differences observed in the yield of the oil of individuals originating from BAF and BDF may be due to the climatic characteristics of these environments, but also to intrinsic factors to the plant. Several authors have already reported intra- and interspecific variations in volatile oil content as a consequence of the influence of abiotic and biotic factors (Douglas et al. 2004; de Oliveira et al. 2005).

In general, the chemical composition of the essential oil of *M. loranthifolia* was similar in the two collection areas, with a predominance of sesquiterpene hydrocarbons. However, some qualitative and quantitative differences were found. The main constituent of the oil of *M. loranthifolia* collected in BAF, for example, (*E*)–caryophyllene, with 47.8% of abundance, which makes this oil a natural source of this sesquiterpene. (*E*)–Caryophyllene was also identified in the oil of *M. loranthifolia* collected in BDF, but in a lower proportion (15.59%).

According to the literature, (*E*)–caryophyllene has been identified in some *Myrcia* species (Henriques et al. 1997; Pereira et al. 2010; Alves et al. 2016; Rosa et al. 2016), notably in *Myrcia splendens* (Sw.) DC. with 45.8% of relative abundance (Jerônimo et al. 2021). Species from other families such as *Teucrium polium* L. (Lamiaceae) with 52.0%, *Tabernaemontana catharinensis* A.DC. (Apocynaceae) with 56,8%, and *Piper nigrum* L. (Piperaceae) with up to 70.4% are great sources of (*E*)–caryophyllene (Orav et al. 2004; Bezić et al. 2011; Boligon et al. 2013). Based on such literature data, the essential oil of *M. loranthifolia* from BAF constitutes one of the largest sources of (*E*)–caryophyllene yet unexplored.

Several biological activities have been described for (*E*)–caryophyllene, namely, anesthetic, analgesic, anticancer, antimicrobial, antioxidant, anti-inflammatory, cardioprotective, hepatoprotective,

gastroprotective, nephroprotective, and immunomodulatory (Gertsch et al. 2008; Hartsel et al. 2016; Machado et al. 2018). *β*–Caryophyllene also enhances anticancer activity of *α*–humulene (Legault and Pichette 2007), another sesquiterpene identified in the two areas under study. According to some authors, *β*–Caryophyllene is a selective CB2 receptor agonist (Gertsch et al. 2008; Klauke et al. 2014; Jha et al. 2021). Therefore, the oil of *M. loranthifolia* plants growing in BAF, which is rich in *β*–Caryophyllene, is promising for future bioassays on the endocannabinoid system.

Germacrene D was the second predominant constituent in the oil of *M. loranthifolia* collected in BAF (10.0%), and it was identified in similar proportion in the oil of *M. loranthifolia* from BDF (10.2%). Germacrene D has been identified in similar concentration (10.31%) in essential oils of *M. rufipilai* McVaugh (Pereira et al. 2010). *Cis*–calamenene was the second major constituent in the oil of *M. loranthifolia* collected in BDF (11.4%), but it was not detected in the oil of individuals from BAF. *Cis*–calamenene has been identified in high amounts (30.1%) in essential oils of *M. sylvatica* (G.Mey.) DC. (Zoghbi et al. 2003).

According to Anderson et al. (2018), germacrene D demonstrated cytotoxic potential against both cancerous and non-tumorigenic cells, especially HeLa (human cervical carcinoma) cells. Cytotoxic potential against MCF-7 human breast cancer cells and antimalarial activity have also been reported (Afoulous et al. 2011). *Cis*–calamenene has also demonstrated larvicidal activity against *Aedes aegypti*, the vector of dengue fever (Muturi et al. 2020). The biological activities reported in the literature for germacrene D and *cis*–calamenene, increase the potential uses of the essential oil of *M. loranthifolia*.

In conclusion, the leaves of *M. loranthifolia* collected in two Brazilian phytogeographic domains concentrate different essential oil contents and have a peculiar chemical composition. Individuals collected in BDF, for example, had higher oil content with a more diverse chemical composition. In turn, those from BAF showed a lower oil content, but with constituents dominating almost 50% of the oil composition. (*E*)–caryophyllene and *cis*–calamenene were the major constituents, but differed quantitatively in plants from the two collection areas, indicating that abiotic factors and/or factors intrinsic to the plant influence the oil composition. The various biological activities reported in the literature for some of the constituents identified in the oil of *M. loranthifolia*, notably for (*E*)–caryophyllene, makes the species interesting for pharmacological studies, including that related to the phytocannabinoid system.

Declarations

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Author contributions RHGS, MTSC and AFMO conceptualized and designed the experiments; RHGS, MHM, JCROFA and DMAFN carried out the experiment and analyzed the data; RHGS and MTSC drafted

and revised the manuscript; RHGS and AFMO critically revised the manuscript. All authors have read and approved this manuscript.

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Data availability The data are available on request.

Conflict of interest The authors declare that there is no conflict of interests.

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Figures

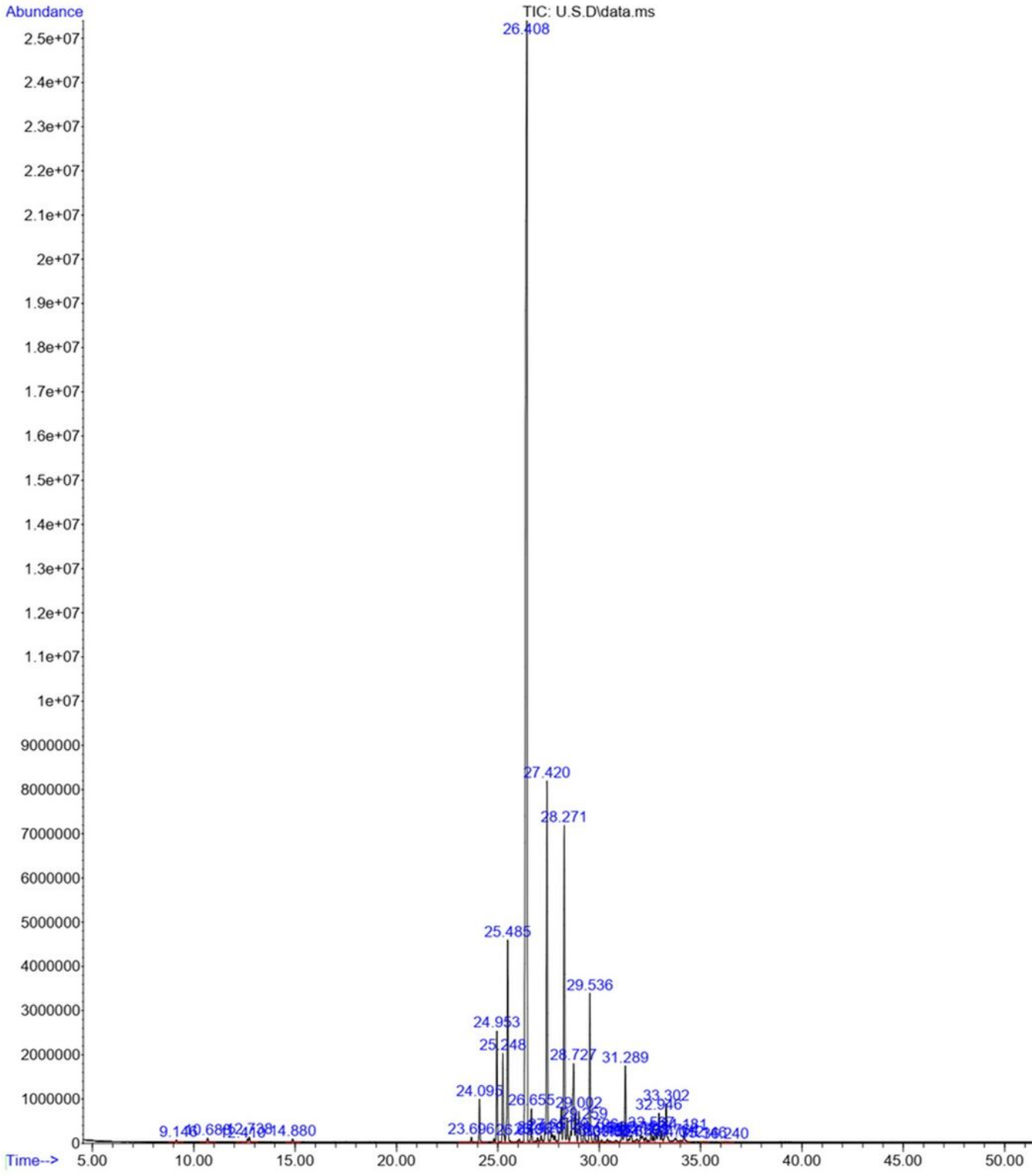

Figure 1

Total ion chromatogram (TIC) of the essential oil from Myrcia loranthifolia leaves occurring in Brazilian Atlantic Forest. The main peak corresponds to (*E*)–caryophyllene (Rt = 26.408 min)

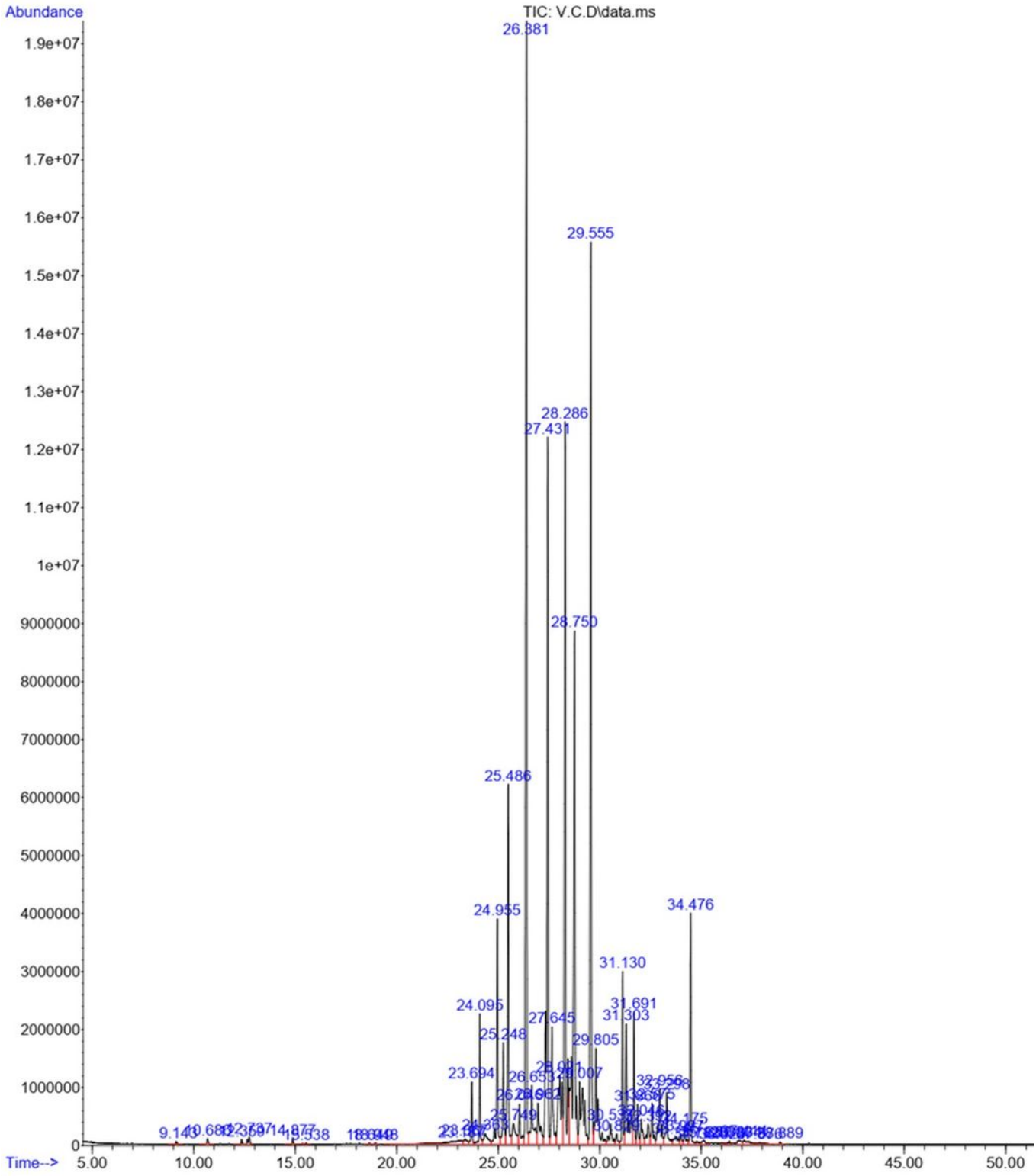

Figure 2

Total ion chromatogram (TIC) of the essential oil from Myrcia loranthifolia leaves occurring in Brazilian Dry Forest. The main peaks correspond to (*E*)–caryophyllene (Rt = 26.381) and *cis*–calamenene (Rt = 29.555 min)