



Seasonal and Circadian Rhythm of a 1,8-Cineole Chemotype Essential Oil of *Calycolpus goetheanus* From Marajó Island, Brazilian Amazon

Natural Product Communications
Volume 15(6): 1–9
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1934578X20933055
journals.sagepub.com/home/npx



Edimara L. dos Santos^{1*}, Ananda M. Lima^{1*}, Vilmara Fabrícia dos S. Moura², William N. Setzer^{3,4}
, Joyce Kelly R. da Silva⁵, José Guilherme S. Maia⁶, João da Silva Carneiro⁷, and
Pablo Luis B. Figueiredo⁷ 

Abstract

Chemical composition of essential oils (EOs) of *Calycolpus* genus have been reported in the literature. However, there is only 1 report about volatile profile from *Calycolpus goetheanus*. This work aims to evaluate the seasonal and circadian influences on EO composition and yield of *C. goetheanus* collected in Marajó Island, Brazilian Amazon. For the circadian study, the leaves were collected in January (rainy season) and July (dry season) every 3 hours during a period of 15 hours. The EOs were obtained by hydrodistillation and their chemical compositions analyzed by gas chromatography coupled to mass spectrometry and flame ionization detectors. The major compound identified in all EO samples was 1,8-cineole with amounts ranged from 14.4% (January, 6 AM) to 33.0% (July, 3 PM). The highest average of 1,8-cineole was obtained during circadian study of the dry season ($25.5\% \pm 5.8\%$) and the lowest during the circadian study of the rainy season ($15.6\% \pm 1.5\%$). The multivariate analysis grouped the samples into 2 different groups: Group I characterized by the lowest amounts of 1,8-cineole (16.0%-18.7%), and Group II characterized by a higher content of 1,8-cineole (24.0%-33.0%). The oil yield and chemical composition did not show relationship with climate parameters (solar radiation, humidity, and temperature). Therefore, there was only quantitative variability in the EOs compositions during the circadian rhythm evaluated on dry and rainy seasons.

Keywords

Myrtaceae, essential oil, seasonality, oxygenated monoterpenoids, multivariate analysis

Received: April 3rd, 2020; Accepted: May 19th, 2020.

Calycolpus goetheanus (Mart. ex DC.) O. Berg [syn. *Calycolpus glaber* (Benth.) O. Berg, *Calycolpus ovalifolius* O. Berg, *Calycolpus schomburgkianus* O. Berg, *Calycolpus angustifolius* L. Riley, *Calycolpus cordatus* L. Riley, *Compomanesia goetheana* O. Berg. ex Hemsl., *Compomanesia glabra* Benth., *Myrtus goetheana* Mart. ex DC., and *Eugenia maranbaensis* G. Don] is a native and nonendemic species from Brazil, commonly known as Goiaba-araçá. It occurs in the Brazilian biomes as Amazon (Pará and Amazonas states) and in the Cerrado (Maranhão state).¹

This species is characterized by shrub or arboreal size, inhabiting water courses or dry land forest as in savannas and in the Amazon. This plant is almost completely glabrous, except for some floral characters like bracts (inner face of sepals and petal margins). Its leaves usually have visible tertiary and marginal ribs, and its flowers have calyx with conspicuous appendages, foliaceous, whose greatest width is positioned in the median region of its lengths. From the morphological

¹Universidade do Estado do Pará campus Salvaterra, PA, Brazil

²Universidade Estadual Vale do Acaraú – Sobral, CE, Brazil

³Department of Chemistry, University of Alabama in Huntsville, USA

⁴Aromatic Plant Research Center, Lehi, UT, USA

⁵Programa de Pós-Graduação em Biotecnologia, Universidade Federal do Pará, Belem, PA, Brazil

⁶Programa de Pós-Graduação em Química, Universidade Federal do Maranhão, Sao Luis, MA, Brazil

⁷Departamento de Ciências Naturais, Centro de Ciências Sociais e Educação, Universidade do Estado do Pará, Belem, PA, Brazil

*The authors Edimara L. dos Santos and Ananda M. Lima contributed equally to the work.

Corresponding Authors:

William N. Setzer, Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA.

Email: wsetzer@chemistry.uah.edu

Pablo Luis B. Figueiredo, Departamento de Ciências Naturais, Centro de Ciências Sociais e Educação, Universidade do Estado do Pará, Belem, PA 66050-540, Brazil.

Email: pablo.figueiredo@uepa.br



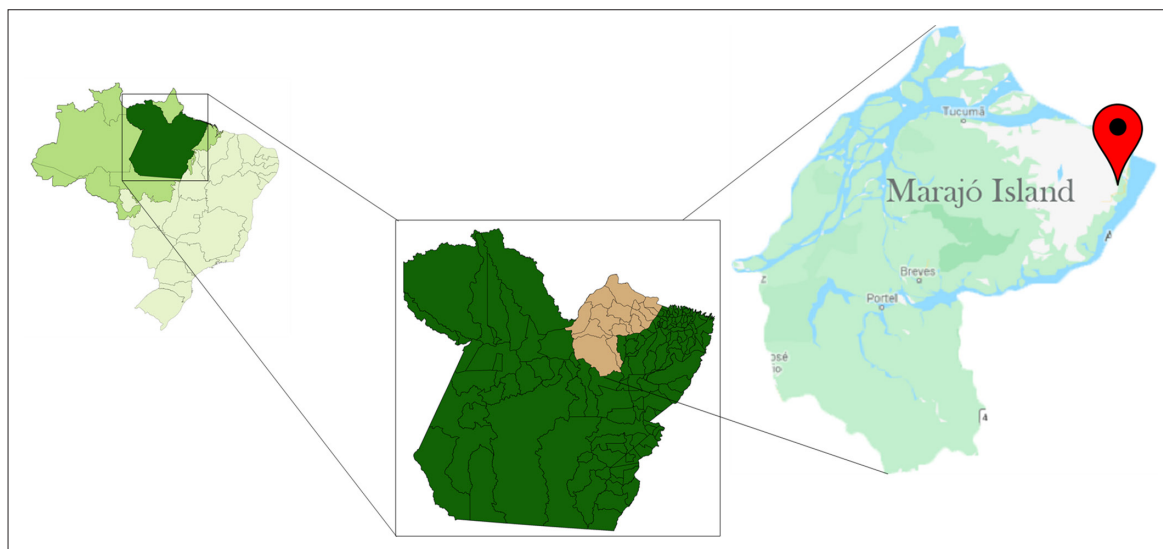


Figure 1. *Calycolpus goetheanus* collection site.

point of view, *C. goetheanus* is very close to *Calycolpus roraimensis* and *Calycolpus revolutus*.¹

The chemical analysis of essential oils (EOs) obtained from *C. goetheanus* has been the subject of only 1 study and reported the occurrence of 1,8-cineole chemotype with predominance of other monoterpenes and sesquiterpenes.² Based on the few reports, the present study aimed to evaluate the seasonal and circadian rhythms influences in the yield and chemical composition of the *C. goetheanus* EO from Marajó island (Amazon), to contribute to the knowledge of its chemotaxonomy.

Results and Discussion

The plant material was collected in Salvaterra (Marajó Island, Brazilian Amazon) (Figure 1). The plant was monitored during an entire day of the months of January (rainy Amazon season) and July (dry Amazon season). For more information, see the Materials and Methods section.

To evaluate the differences in environmental conditions between January and July, the climate data rainfall, relative air humidity, and solar radiation were obtained from the website of the Instituto Nacional de Meteorologia (INMET, <http://www.inmet.gov.br/portal/>) of the Brazilian Government.

Table 1. Correlation Between the *Calycolpus goetheanus* Oil Yields and the Environmental Conditions.

Environmental factors	Correlation coefficients (R)	
	January	July
Humidity	-0.05	0.20
Solar radiation	0.18	0.03
Temperature	-0.32	-0.01

In January, the rainfall index (153.0 mm) was 4 times greater than in the dry period (35.8 mm). Moreover, the relative air humidity was 81.14% in January and 76.46% in July. Additionally, the solar radiation was higher in July (951.58 kJ/m²) than in January (747.54 kJ/m²). Thus, taking into account the climate parameters, the month of January belongs to rainy season and July to dry season.

The Brazilian Amazonian climate is characterized only by dry and rainy seasons. Due to the permanent humid and warm climate, the Amazon presents spatial and seasonal heterogeneity of rainfall. Based on the precipitation data of 2018, the rainy season occurred from December to April and the dry season from June to November. May was a period of transition between these 2 seasons.³

In the circadian rhythm of the oil yield of *C. goetheanus* carried out in July (dry season), the yields ranged from 1.2% (3 PM) to 2.4% (9 AM), with mean of 1.8% ± 0.4% during all day long. While in January (rainy season), it varied between 1.8% (9 AM) and 2.3% (6 AM) with mean of 2.2% ± 0.2%. Thus, there was no statistical difference between the means of yields from dry (1.8% ± 0.4%) and rainy (2.1% ± 0.2%) seasons in the Tukey test ($P > .05$).

The EO yields from the circadian collections of rainy and dry months showed low correlation with the climate parameters: humidity, solar radiation, and temperature (Table 1). In summary, Pearson's correlation analysis shows no correlation between quantitative parameters of in *C. goetheanus* EO and environmental conditions.

A previous analysis of *C. goetheanus* sampled in the municipality of Maracanã, state of Pará, Brazil, showed an oil yield of 1.0%.² Also, the species *Calycolpus moritzianus* collected in

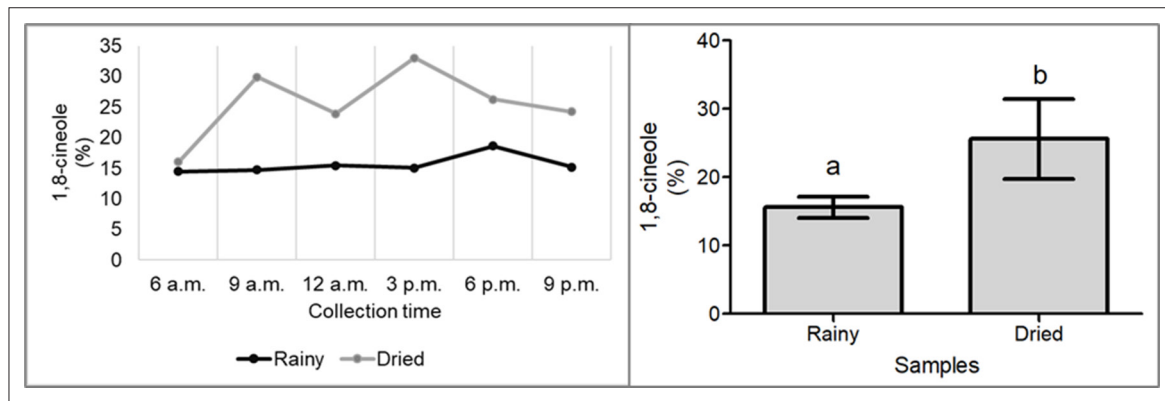


Figure 2. Circadian rhythm of 1,8-cineole content (left) and average during the collections of rainy and dry seasons (right).

Venezuela and Colombia presented the oil yields of 0.08% and 1.0%, respectively.^{4,5}

The constituents of the oils were identified and quantified by gas chromatography coupled to mass spectrometry (GC-MS) and flame ionization detector (GC-FID), respectively. A total of 101 compounds were identified, representing an average of 95.3% of the composition of the total oils (Table 2). Oxygenated monoterpenes were predominant (20.4%-42.8%) followed by sesquiterpene hydrocarbons (16.8%-46.5%), oxygenated sesquiterpenes (13.9%-36.5%), and monoterpene hydrocarbons (6.6%-14.9%). The main constituents were 1,8-cineole (14.5%-33.0%) and limonene (5.4%-11.7%);

followed by α -terpineol (3.5%-7.9%); and α -copaene (3.5%-7.3%), (*E*)-caryophyllene (0.0%-4.9%), and δ -cadinene (0.0%-9.9%).

The oxygenated monoterpene 1,8-cineole was the main compound identified in the samples with amount ranged from 14.4% (January, 6 AM) to 33.1% (July, 3 PM). Comparing the average amount from 2 circadian rhythms, the 1,8-cineole content was higher in July (25.5% \pm 5.8%, dry period) than in January (15.6% \pm 1.5%, rainy season) and displayed a significant statistical difference in Tukey test ($P < .05$) (see Figure 2).

The results of Figure 2 suggest that the quantitative variation in the chemical composition of the circadian rhythm could

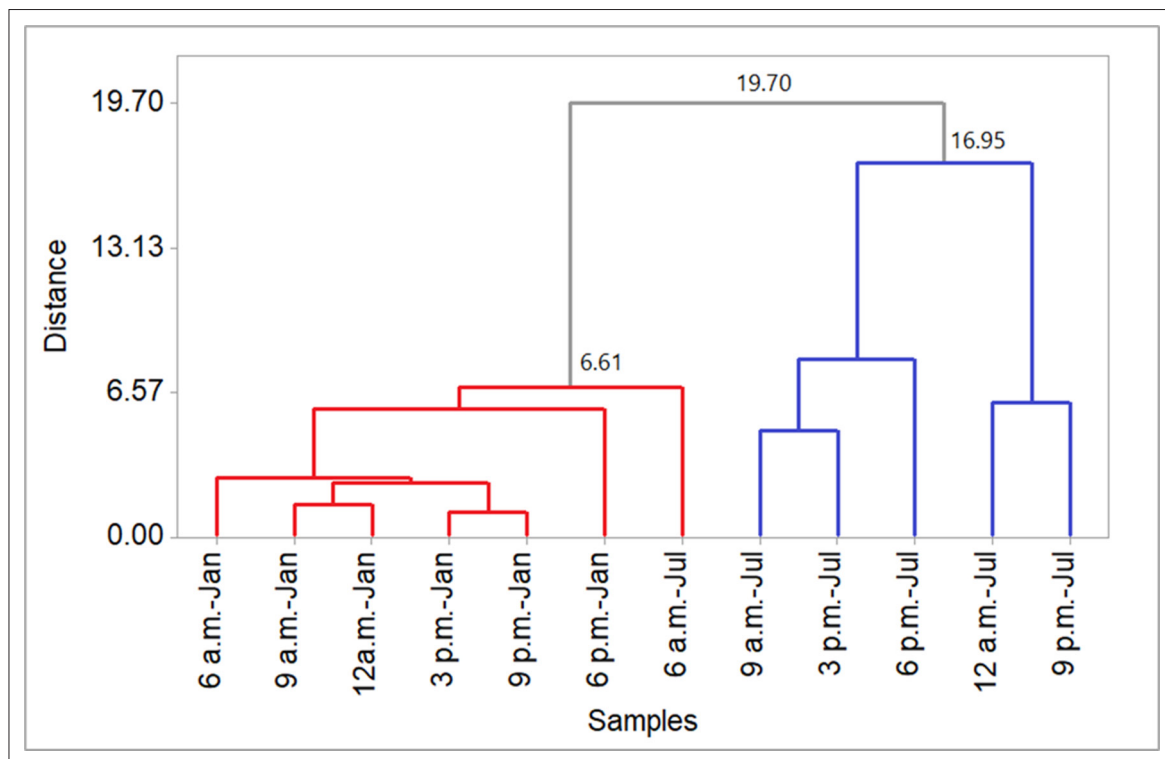


Figure 3. Hierarchical cluster analysis of essential oils sampled in the circadian rhythms made with Euclidean distance and complete linkage.

Table 2. Essential Oil Yield (%) and Constituents (%) of *Calycolpus goetbeanus* Sampled in Rainy and Dry Seasons.

Constituents	IR _(C)	IR _(L)	Oil yield (%)											
			January—rainy season						July—dry season					
			6 AM	9 AM	12 AM	3 PM	6 PM	9 PM	6 AM	9 AM	12 AM	3 PM	6 PM	9 PM
			2.3	1.8	2.0	2.2	2.0	2.3	1.3	2.4	1.8	1.2	1.9	1.9
Hexanal	796	801 ^a		tr	tr									
(2 <i>E</i>)-Hexenal	844	846 ^a		0.1	tr	tr								
(3 <i>Z</i>)-Hexenol	847	850		0.2	tr									
(2 <i>E</i>)-Hexenol	857	854 ^a		tr										
<i>n</i> -Hexanol	860	863 ^a		tr	tr	tr								
α -Pinene	933	932 ^a	1.0	1.1	1.0	0.7	0.9	0.9	0.4	1.0	0.5	1.1	0.9	0.6
α -Fenchene	946	945 ^a			tr									
Camphene	948	946 ^a	0.1	0.1	0.1	0.1	0.1	0.1	tr	0.1		0.1	0.1	
Myrcene	990	988 ^a	0.6	0.8	0.7	0.6	0.6	0.6	0.4	0.8	0.2	1.0	0.7	0.2
<i>n</i> -Decane	999	1000 ^a		tr	tr			tr						
α -Phellandrene	1005	1002 ^a	0.1	0.1	0.1	0.1		0.1		tr		0.1		
α -Terpinene	1016	1014 ^a	0.2	0.2	0.2	0.2		0.2		tr		0.1	0.1	
<i>p</i> -Cymene	1024	1020 ^a	0.3	0.5	0.4	0.2	0.7	0.3	0.4	0.8	0.6	0.8	0.6	0.7
Limonene	1029	1024^a	7.9	6.5	7.5	6.6	8.0	7.5	6.0	8.6	5.4	11.7	10.0	5.8
1,8-Cineole	1032	1026^a	14.5	14.8	15.4	15.1	18.7	15.2	16.0	29.9	24.0	33.0	26.2	24.3
(<i>E</i>)- β -Ocimene	1046	1044 ^a	tr	tr	0.1	tr		0.1				tr		
γ -Terpinene	1057	1054 ^a	0.3	0.3	0.3	0.4	0.1	0.3	0.1	0.1		0.1	0.1	
Terpinolene	1088	1086 ^a	0.3	0.3	0.3	0.3	0.1	0.3	0.1	0.1		0.2	0.1	
Linalool	1099	1095 ^a	0.8	0.9	0.9	0.8	0.9	0.9	0.6	1.0	1.3	1.5	1.0	0.7
<i>endo</i> -Fenchol	1113	1114 ^a		tr										
<i>cis</i> -Limonene oxide	1132	1132 ^a									0.2			
Borneol	1165	1165 ^a	0.2	0.2	0.2	0.2	0.2	0.2			0.3		0.1	0.2
Terpinen-4-ol	1176	1174 ^a	0.6	0.6	0.6	0.5	0.6	0.6	0.3	0.7	0.6	0.7	0.5	0.2
α-Terpineol	1190	1186^a	5.4	5.0	5.2	4.9	5.6	5.0	3.5	7.2	7.9	7.6	5.7	3.5
<i>trans</i> -Carveol	1217	1215 ^a									0.1			
Carvone	1242	1239									0.2			0.2
Cyclosativene	1367	1369 ^a	0.7	0.8	0.7	0.6	0.5	0.6	0.9	0.3	0.7	0.3	0.4	0.8
α -Ylangene	1371	1373 ^a	0.6	0.6	0.5	0.5	0.4	0.4	0.6	0.3	0.4	0.2	0.3	0.2
α-Copaene	1375	1374^a	5.9	5.0	4.8	4.6	4.4	4.4	7.4	4.1	5.0	3.6	5.3	3.5
Sativene	1389	1390 ^a	0.3	0.3	tr	0.2	0.2	0.2	0.3	0.2	0.3	0.1	0.2	0.3
β -Longipinene	1406	1400 ^a		tr	tr	tr								
α -Gurjunene	1409	1409 ^a	0.5	0.4	0.4	0.4	0.3	0.4	0.4	0.2		0.2	0.2	
α -Cedrene	1411	1410 ^a	tr	0.1	tr	tr		tr	tr					
(<i>E</i>)-Caryophyllene	1419	1417^a	4.8	4.4	4.3	4.3	4.0	4.4	4.9	4.0	0.6	3.3	4.3	
Carvone hydrate	1420	1422 ^a									0.2			
γ -Maaliene	1423	1430 ^b	0.1	0.1	0.1	0.1		tr						
<i>trans</i> - α -Bergamotene	1435	1432 ^a	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1			0.1	
α -Guaiene	1438	1437 ^a	0.1	0.1	0.1	0.1	0.1	0.1	0.1					
6,9-Guaiadiene	1442	1442 ^a	1.3	1.2	1.1	1.1	0.8	1.1	1.1	0.6		0.5	0.8	
Isogermacrene D	1448	1447 ^b	0.7	0.7	0.6	0.6	0.5	0.6	0.6			0.7		
<i>cis</i> -Muurola-3,5-diene	1450	1448 ^a	0.6	0.5	0.5	0.5	0.3	0.5		0.2			0.3	
<i>trans</i> -Muurola-3,5-diene	1450	1451 ^a							0.4			0.2		
α -Humulene	1452	1452	1.3	1.3	1.2	1.2	1.2	1.3	1.3	1.0	0.3	0.8	1.1	
(<i>E</i>)- β -Farnesene	1455	1454 ^a	0.5	0.5	0.4	0.4	0.3	0.4	0.5	0.3		0.2	0.3	
9- <i>epi</i> -(<i>E</i>)-Caryophyllene	1460	1458 ^a	0.4	0.4	0.3	0.3	0.2	0.3	0.3	0.1	0.1	0.1	0.2	
4,5-Di- <i>epi</i> -aristolochene	1468	1471 ^a	0.1	0.1	0.1	0.1	0.1	0.1	0.1		0.1			

(Continued)

Table 2. Continued

Constituents	IR _(C)	IR _(L)	Oil yield (%)											
			January—rainy season						July—dry season					
			6 AM	9 AM	12 AM	3 PM	6 PM	9 PM	6 AM	9 AM	12 AM	3 PM	6 PM	9 PM
			2.3	1.8	2.0	2.2	2.0	2.3	1.3	2.4	1.8	1.2	1.9	1.9
<i>trans</i> -Cadina-1(6),4-diene	1472	1472 ^a	0.4	0.3	0.4	0.4		0.4						tr
γ-Gurjunene	1475	1475 ^a												0.9
Selina-4,11-diene	1474	1476 ^b	0.9	1.3	1.1	1.1	0.6	1.0	1.4	0.7	0.9	0.5	0.7	
Amorpha-4,7(11)-diene	1475	1479 ^a	0.4		0.7		0.2							
α-Amorphene	1478	1482 ^b	0.7	0.7		0.7	0.4	0.6	0.6	0.3	0.2	0.2	0.4	
α-Curcumene	1481	1479	0.3	0.5	0.3	0.2	0.5	0.3	0.6	0.4	0.7	0.3	0.4	0.8
β-Selinene	1485	1489 ^a	3.4	3.3	3.1	3.3	3.1	3.2	4.4	3.4	4.9	2.3	2.9	5.2
Viridiflorene	1487	1496 ^a	0.6	0.5	0.4	0.5	0.4	0.5						
δ-Selinene	1490	1492 ^a	0.7	0.6	0.6	0.7	0.3	0.6	0.5	0.2		0.2	0.3	
α-Selinene	1494	1498 ^a	2.7	2.6	2.4	2.6	2.2	2.4	2.8	2.1	0.9	1.6	2.0	0.2
<i>trans</i> -Muuroala-4(14),5-diene	1497	1493 ^a	1.0	0.9	0.8	0.9	0.5	0.8	0.7			0.4		
<i>epi</i> -Zonarene	1497	1500 ^a								0.4			0.5	
α-Muurolene	1499	1500 ^a	1.1	1.0	1.0	0.9	0.8	0.9	1.3	0.6	0.7	0.5	0.8	0.7
δ-Amorphene	1506	1511 ^a	2.2	1.2	1.8	2.3		2.7						
β-Bisabolene	1507	1505 ^a	1.1	1.2	0.9	0.7	1.1	0.3	2.2	1.0	0.9	0.9	1.4	0.6
β-Curcumene	1510	1511 ^b	0.4	0.3	0.3	0.4			0.1				tr	
γ-Cadinene	1513	1512 ^b	1.5	1.4	1.4	1.4	1.2	1.4		0.9	1.4	0.7	1.1	1.6
7- <i>epi</i> -α-Selinene	1516	1520 ^a	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1			0.1	
<i>trans</i> -Calamenene	1521	1521 ^a									0.7			
δ-Cadinene	1523	1522^a	8.2	6.8	7.1	7.4	6.1	7.4	9.9	5.5		5.3	7.4	
Zonarene	1525	1528 ^a	0.5	0.5	0.6	0.6	0.3	0.5						
<i>trans</i> -Cadina-1,4-diene	1531	1533 ^a	0.2	0.1	0.2	0.2		0.1	0.1					
Selina-4(15),7(11)-diene	1534	1540 ^b	0.1	0.1	0.1	0.1	0.1	0.1	0.1					
α-Cadinene	1536	1537 ^a	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1		0.1	0.1	
α-Calacorene	1541	1544 ^a	1.9	1.7	1.8	1.9	1.7	2.0	1.7	0.9		0.9	1.2	
β-Calacorene	1564	1561 ^a							0.4					
Palustrol	1566	1567 ^a	0.2	0.2	0.1	0.2	0.1	0.2						
Caryolan-8-ol	1571	1576 ^a							0.1	0.8			1.0	
Caryophyllenyl alcohol	1568	1582 ^a	0.9	1.0	0.9	1.0	1.1	1.1	1.0		1.4	0.7		2.5
Caryophyllene oxide	1582	1582 ^a	0.4	0.5	0.4	0.4	0.8	0.5	0.6	0.4	6.4		0.3	9.5
Globulol	1586	1590 ^a	0.5	0.5	0.6	0.6	0.5	0.6	0.3	0.3	0.8	0.3	0.3	
Guaiol	1590	1600 ^a										0.7		
Viridiflorol	1591	1592 ^a	0.8	1.0	1.0	1.0	1.1	1.0	0.8	0.8	0.3	0.3	0.7	1.2
Cubeban-11-ol	1595	1592 ^a										0.2		0.5
Ledol	1599	1602 ^a	0.7	0.9	0.9	0.9	1.0	0.9						
Carotol	1602	1601 ^a	1.4	1.6	1.6	1.7	1.9	1.7	0.7	1.5	3.5	1.5		3.3
Humulene epoxide II	1606	1608 ^a					0.2		0.1		1.1			1.6
Rosifoliol	1606	1609 ^b		0.1	0.1	0.1								
Copaborneol	1611	1613 ^b	1.0	1.2	1.2	1.3	1.4	1.2			2.3			2.2
1,10-Di- <i>epi</i> -cubenol	1613	1618 ^a	0.6	0.7	0.7	0.7	0.8	0.7	0.6	0.6	0.7	0.5	0.5	0.4
Junenol	1616	1618 ^a	0.5	0.7	0.7	0.8	0.9	0.8	0.5	0.5	0.3	0.5	0.5	0.2
<i>iso</i> -Longifolol acetate	1620	1620 ^a							0.2					
1- <i>epi</i> -Cubenol	1626	1627 ^a	0.8	0.9	1.0	1.1	1.1	1.1	0.8	0.7	1.0	0.7	0.8	0.9
γ-Eudesmol	1629	1630 ^a	0.6	0.8	0.8	0.9	0.9	0.8	0.6	0.5	0.5	0.4	0.5	0.4

(Continued)

Table 2. Continued

Constituents	IR _(C)	IR _(L)	Oil yield (%)											
			January—rainy season						July—dry season					
			6 AM	9 AM	12 AM	3 PM	6 PM	9 PM	6 AM	9 AM	12 AM	3 PM	6 PM	9 PM
<i>epi</i> - α -Cadinol (τ -cadinol)	1638	1638 ^a	2.3	1.8	2.0	2.2	2.0	2.3	1.3	2.4	1.8	1.2	1.9	1.9
<i>epi</i> - α -Muurolol (τ -muurolol)	1639	1640 ^a	0.7	0.8	0.8	0.9	1.0	0.9	0.9	0.6	0.8	1.1	0.6	0.8
α -Muurolol (torreyol)	1644	1644 ^a	0.7	0.8	0.9	0.9	1.1	0.9	0.8	0.6	0.8		0.7	0.9
β -Eudesmol	1647	1649 ^a	0.6	0.7	0.7	0.8	1.2	0.8	0.5	0.5	0.9	0.4	0.4	1.1
Pogostol	1652	1651 ^a	2.4	3.1	3.1	3.5	4.1	3.6	2.9	3.3	5.5	2.4	2.7	5.0
<i>cis</i> -Calamene-10-ol	1655	1660 ^a	0.1	0.2		0.2	0.3	0.2	0.2	0.1	0.5			0.7
<i>trans</i> -Calamene-10-ol	1662	1668 ^a	0.1	0.5		0.1	0.2	0.2	0.7		0.3			0.7
<i>epi</i> - β -Bisabolol	1666	1670 ^a	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1		0.2	0.1	
β -Bisabolol	1668	1674 ^a	0.2	0.3	0.3	0.3	0.3	0.3	0.2	0.2				
Cadalene	1671	1675 ^a	0.1	0.1	0.1	0.1	0.2	0.1	0.1		1.0		0.2	2.0
Mustakone	1673	1676 ^a									0.1			1.0
<i>epi</i> - α -Bisabolol	1680	1683 ^a	3.8	3.9	4.0	4.6	4.9	4.8	4.6	4.4	4.3	3.8	4.5	2.6
α -Bisabolol	1681	1685 ^a	0.7	1.0	0.9	1.0	1.2	1.0	0.8	0.7	0.7	0.6	0.8	0.4
Germacra-4(15),5,10(14)-trien-1 α -ol	1687	1685 ^a												0.2
10- <i>nor</i> -Calamene-10-one	1702	1696												0.1
Juniper camphor	1691	1696 ^b	0.1	0.1	0.1	0.1	0.1	0.1	0.1	tr				
(2 <i>E</i> ,6 <i>Z</i>)-Farnesol	1716	1714 ^a	0.1	0.1	0.1	0.1		0.1	0.1					
<i>n</i> -Hexadecanol	1875	1874 ^a	0.1	0.3	0.2	0.1		0.2	0.7	0.2	0.3	0.1	0.2	0.6
Heneicosane	2100	2107 ^a							0.1					
<i>E,E</i> -Geranyl linalool	2026	2026 ^a	0.2	0.4	0.3	0.3	0.2	0.4	0.7	0.2	0.1	0.1	0.2	
Monoterpene hydrocarbons			10.8	9.8	10.7	9.2	10.4	10.3	7.3	11.6	6.6	14.9	12.4	7.2
Oxygenated monoterpenoids			21.5	21.5	22.4	21.4	26.0	21.9	20.4	38.7	34.8	42.8	33.6	29.0
Sesquiterpene hydrocarbons			46.5	42.1	40.8	41.9	33.6	40.4	45.8	27.4	19.6	24.0	32.2	16.8
Oxygenated sesquiterpenoids			18.4	22.1	21.5	23.6	26.3	23.9	18.5	16.9	32.5	13.9	14.7	36.5
Others			0.4	1.0	0.5	0.5	0.2	0.5	1.5	0.3	0.4	0.2	0.4	0.6
Total identified			97.6	96.4	95.9	96.5	96.6	96.9	93.4	95.3	94.0	96.6	93.8	90.2

RI_(C), calculated retention index; RI_(L), literature retention index; tr, trace (<0.05%).

Bold indicates main constituents (above 5%).

^aAdams.⁶

^bMondello.⁷

be attributed to the climatic changes, with a strong correlation observed in January. Also, it is possible to see in Table 3 the correlation with the climatic factors: a strong positive correlation between the temperature and the monoterpene hydrocarbons; a significant negative correlation of the sesquiterpene hydrocarbons and the solar radiation; and a positive correlation between the oxygenated sesquiterpenes and the solar radiation. These results were obtained by Pearson's correlation coefficient analysis, involving the compound classes of the oils and the climatic parameters.

It is known that, in addition to environmental factors, differences in oil yield and composition can be attributed to

genetic, geographical, and invasive predators (herbivory and pathogens).⁸ For example, some Myrtaceae species have shown qualitative and quantitative variability in their EO compositions according to different collection sites. Oils of *Eugenia biflora* presented 3 chemotypes: (1) caryophyllane-type, with (*E*)-caryophyllene and caryophyllene oxide; (2) cadinane-type, characterized by α -cadinol; and (3) aromadendrane-type, with predominance of globulol, germacrene B, and γ -elemene⁹; oils of *Eugenia uniflora*, which were classified into 4 chemotypes: (1) selin-1,3,7(11)-trien-8-one and selin-1,3,7(11)-trien-8-one epoxide; (2) selin-1,3,7(11)-trien-8-one, selin-1,3,7(11)-trien-8-one epoxide, and caryophyllene oxide; (3) curzerene,

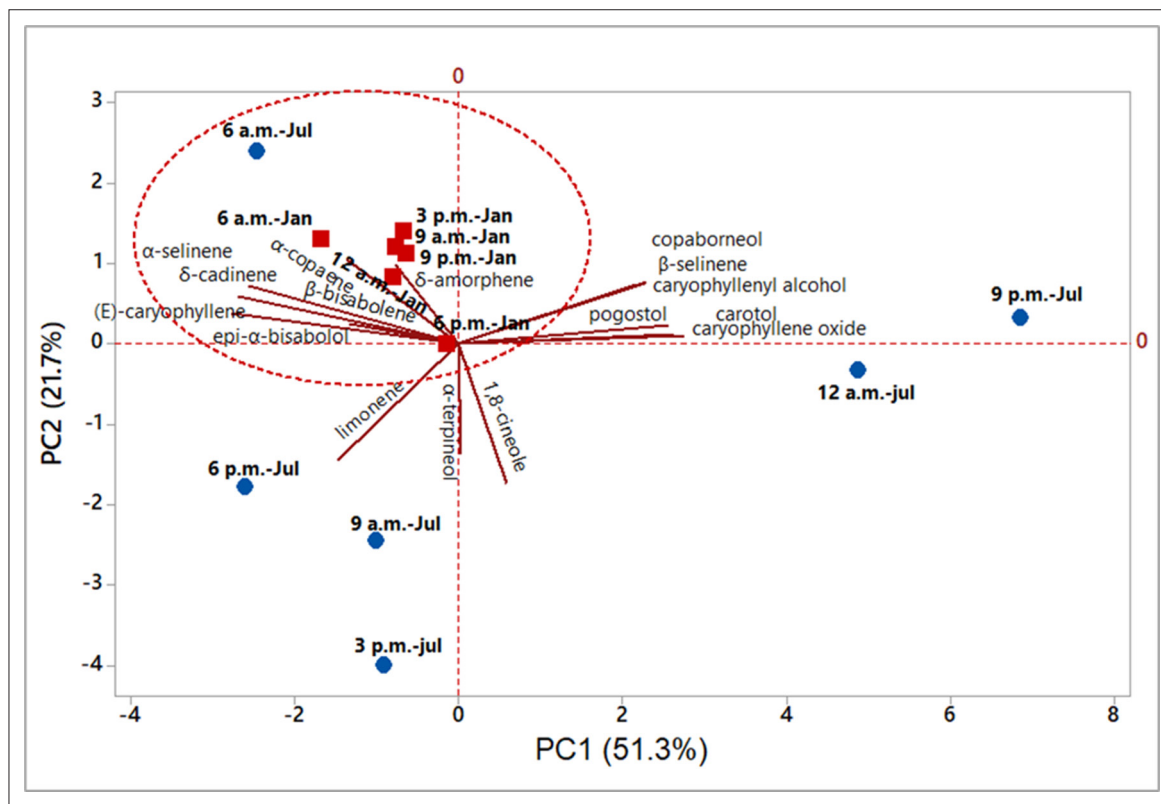


Figure 4. Principal component analysis of chemical compositions of essential oils sampled in a daily cycle of the rainy season (January) and dried season of *Calycolpus goetheanus*.

germacrene B, and germacrene; and (4) germacrene B, curzerene, and (*E*)-caryophyllene.¹⁰ Oils of *Psidium guineense*, which were grouped into 3 chemotypes: (1) α -pinene and limonene; (2) β -bisabolene and *epi*- β -bisabolol; and (3) (*E*)-caryophyllene and caryophyllene oxide.¹¹

The EO of *E. uniflora* collected in north of Brazil did not display seasonal variation in oil yield or chemical composition. Environmental factors also had little influence on the composition, displaying curzerene as the principal constituent, with no significant difference between dry ($42.7\% \pm 6.1\%$) and rainy ($40.8\% \pm 5.9\%$) seasons.³ On the other hand, *Myrcia*

syhatica presented a quantitative variation in its composition attributed to climatic changes, with positive correlation of the temperature and solar radiation with the concentrations of oxygenated sesquiterpenoids and negative correlation with the sesquiterpene hydrocarbons.¹²

In order to evaluate the chemical variability during the circadian rhythm, the multivariate analysis by hierarchical cluster analysis (HCA) and principal component analysis (PCA) techniques was applied to the primary constituents present in oils (content $\geq 1.5\%$). The HCA (Figure 3) performed with complete linkage and Euclidean distance showed the formation of

Table 3. Correlation Between Chemical Compositions and the Environmental Conditions.

Main constituent/ compound classes	Correlation coefficients (R)					
	January			July		
	Humidity	Solar radiation	Temperature	Humidity	Solar radiation	Temperature
1,8-Cineole	0.56	0.65	0.29	-0.18	0.26	0.33
Monoterpene hydrocarbons	-0.45	0.13	0.75*	-0.38	0.39	0.44
Oxygenated monoterpenoids	0.48	0.63	0.39	-0.03	0.12	0.17
Sesquiterpene hydrocarbons	-0.53	-0.72*	-0.15	0.09	0.19	-0.25
Oxygenated sesquiterpenoids	0.59	0.75*	-0.14	0.13	-0.09	-0.08
Others	-0.49	-0.45	-0.02	0.17	-0.30	-0.31

*Significant at $P < .10$.

2 different groups with similarity of 19.70%. The groups were classified as follows: Group I characterized by the lowest amounts of 1,8-cineole (16.03%-18.67%), which is formed for the samples collected from daily cycle of the rainy season (January) and only 1 sample from dry season (July) collected at 6 AM. Group II resulted from the grouping of samples collected during 1 day in the dry period (July, except of sample collected at 6 AM) and it was characterized by a higher content of 1,8-cineole (24.0%-33.0%).

These results were confirmed by the PCA (PC1 and PC2, Figure 4) which explained a proportional variance of 51.3% and 22.7%, respectively, representing a total of 73.0% of data variation. In other words, all samples collected from circadian rhythm of the rainy season (January) and 1 sample from dried season (July, 6 AM) were grouped in a little dispersed group. The remaining samples (collected from circadian rhythm of the dried season) formed a scattered group (see Figure 4).

The chemical composition and biological activities of *Calycolpus* EOs have been described in the literature. *Calycolpus moritzianus* oil, sampled in Venezuela, showed predominance of (*E*)-caryophyllene (21.9%), α -pinene (10.9%), and viridiflorol (9.7%), this EO displayed antibacterial activity against *Staphylococcus aureus* and *Enterococcus faecalis*.⁴ The EOs of 40 *C. moritzianus* specimens collected in Colombia showed as the major constituents 1,8-cineol (12.9%-49.8%), limonene (20.0%-47.1%), (*E*)-caryophyllene (1.1%-9.9%), and guaiol (2.0%-12.3%).⁵ The EO of leaves of *Calycolpus warszewiczianus* Berg from Costa Rica showed (*E*)-caryophyllene (30.1%) and caryophyllene oxide (9.8%) as the main constituents.¹³

The monoterpene 1,8-cineole (eucalyptol or 1,8-epoxy-*p*-methane) has a fresh fragrance, camphoraceous features, and a spicy flavor. It is an organic compound naturally found as the main compound in the EOs of many herbs, mainly in the *Eucalyptus* genus.^{14,15} It is widely used as an ingredient in the pharmaceutical and cosmetics industry, for example, in nasal sprays or as a disinfectant as a food flavoring agent.¹⁵ It also showed anti-inflammatory activity, suggesting therapeutic potential as a source for the development of new anti-inflammatory agents, reducing edema induced by histamine and arachidonic acid and granuloma.¹⁶ Moreover, 1,8-cineole has displayed antifungal and antiaflatoxigenic activities against *Aspergillus flavus*, at a concentration of 250 ppm with dramatic downregulation of aflE and aflL gene expression.¹⁷ A minimum lethal dose of 50 mg/kg for a human adult has been proposed, taking into account the toxic effects of 1,8-cineole.¹⁸

Conclusion

The environmental parameters did not show relationship with oil yield and 1,8-cineole content in the *C. goetheanus* EO, as well as displaying only quantitative influence on its chemical composition.

The significant oil yield and higher amounts of 1,8-cineole obtained in the dry period indicates that this plant can be used

as a renewable source of this compound. Due to the little qualitative variation of its EO chemical profile, the standardization and its economic exploration can be viable.

Materials and Methods

Plant Material and Climate Data

A wild specimen of *C. goetheanus* was collected in Salvaterra, Marajó Island, Pará state, Brazil (0°45'19.73"S/48°30'41.89"W). For the circadian study, the leaves were collected in January (rainy season) and July (dry season) at the hours of 6 AM, 9 AM, 12 AM, 3 PM, 6 PM, and 9 PM. Plant identification was performed by comparison with an authentic specimen *C. goetheanus*, and a voucher specimen has been deposited in the Herbarium of Museu Paraense Emílio Goeldi (MG 236265).

Extraction and Chemical Composition Analysis of the EO

The plant material was air-dried (2 days) at room temperature, after collection. Then, it was ground and submitted to hydro-distillation using a Clevenger-type apparatus (3 hours).¹⁹ The oils obtained were dried over anhydrous sodium sulfate and total oil yields were expressed as mL/100 g of the dried material.²⁰ The chemical composition analysis was performed by GC-MS, using a Shimadzu instrument Model QP 2010 ultra (Shimadzu, Tokyo, Japan), equipped with a Rtx-5MS (30 m × 0.25 mm; 0.25 μ m film thickness) fused silica capillary column (Restek, Bellefonte, United States). Helium was used as carrier gas adjusted to 1.0 mL/min at 57.5 kPa; split injection (split ratio 1:20) of 1 μ L of hexane solution (oil 5 μ L:hexane 500 μ L); injector and interface temperature were 250°C; oven temperature programmed was 60°C to 240°C (3°C/min), followed by an isotherm of 10 minutes. Electron Impact Mass Spectrometry (EIMS): electron energy, 70 eV; ion source temperature was 200°C. The mass spectra were obtained by automatic scanning every 0.3 seconds, with mass fragments in the range of 35 to 400 m/z . The compounds present in the samples were identified by comparison of their mass spectrum and retention index calculated for all volatile components using a linear equation of Van Den Dool and Kratz²¹ with data present in commercial libraries as FFNSC 2⁷ and Adams.⁶ Retention indices were calculated using *n*-alkane standard solutions (C8-C40, Sigma-Aldrich, St. Louis, MO, United States) in the same chromatographic conditions. The GC-FID analysis was carried out on a Shimadzu QP-2010 instrument, equipped with FID, in the same conditions, except that hydrogen was used as the carrier gas. The percentage composition of the oil samples was computed from the GC-FID peak areas.

Statistical Analysis

The multivariate analysis was performed by using as variables the EO constituents with content above 1.5%. The data matrix was standardized by subtracting the mean and then dividing it by the standard deviation. For HCA, the

complete linkage method and the Euclidean distance were used.⁹ All analyses were performed using the software Minitab (free 390 version, Minitab Inc., State College, PA, United States).

Acknowledgment

We are grateful to CNPq and CAPES, institutions of support for scientific research of the Brazilian Government.


Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: We are grateful to CNPq and CAPES, institutions of support for scientific research of the Brazilian Government.

ORCID ID

William N. Setzer  <https://orcid.org/0000-0002-3639-0528>

Pablo Luis B. Figueiredo  <https://orcid.org/0000-0003-1365-3513>

References

- Sobral M, Proença C, Souza M, Mazine F, Lucas E. Myrtaceae in Lista de Espécies da flora do Brasil. *Jard Botânico do Rio Janeiro*. 2019
- Pereira RA, Zoghbi MdsGB, Bastos MdeNdoC. Essential oils of twelve species of Myrtaceae growing wild in the sandbank of the Resex Maracanã, state of Pará, Brazil. *J Essent Oil Bear Plants*. 2010;13(4):440-450. doi:10.1080/0972060X.2010.10643847
- da Costa JS, Barroso AS, Mourão RHV, da Silva JKR, Maia JGS, Figueiredo PLB. Seasonal and Antioxidant Evaluation of Essential Oil from *Eugenia uniflora* L., Curzerene-Rich, Thermally Produced In Situ. *Biomolecules*. 2020;10(2):32810.3390/biom10020328 19 Feb 2020. doi:10.3390/biom10020328
- Díaz T, Mora FD, Velasco J, et al. Chemical Composition and In Vitro Antibacterial Activity of the Essential Oil of *Calycolpus Moritzianus* (O. Berg) Burret from Mérida, Venezuela. *Nat Prod Commun*. 2008;3(6):937-940.
- Yáñez-Rueda X, Betancur-Galvis L, Agudelo-Gómez LS, et al. Composición química y actividad biológica de aceites esenciales de *Calycolpus moritzianus* recolectado en el Norte de Santander, Colombia. *Rev la Univ Ind Santander Salud*. 2009;41:259-267.
- Adams RP. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. Allured Publishing Corporation; 2007.
- Mondello L. *FFNSC 2: Flavors and Fragrances of Natural and Synthetic Compounds, Mass Spectral Database*. John Wiley & Sons Inc; 2011.
- Gobbo-Neto L, Lopes NP. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Quím Nova*. 2007;30(2):374-381. doi:10.1590/S0100-40422007000200026
- Figueiredo PLB, Fernandes HA, da Silva ARC, et al. Variability in the Chemical Composition of *Eugenia biflora* Essential Oils from the Brazilian Amazon. *Nat Prod Commun*. 2019;15(12):1-6.
- Figueiredo PLB, Pinto LC, da Costa JS, et al. Composition, antioxidant capacity and cytotoxic activity of *Eugenia uniflora* L. chemotype-oils from the Amazon. *J Ethnopharmacol*. 2019;232:30-38. doi:10.1016/j.jep.2018.12.011
- Figueiredo PLB, Silva RC, da Silva JKR, Suemitsu C, Mourão RHV, Maia JGS. Chemical variability in the essential oil of leaves of Araçá (*Psidium guineense* Sw.), with occurrence in the Amazon. *Chem Cent J*. 2018;12(1. doi:10.1186/s13065-018-0428-z
- Raposo JDA, Figueiredo PLB, Santana RL, et al. Seasonal and circadian study of the essential oil of *Myrcia sylhatica* (G. Mey) DC., a valuable aromatic species occurring in the Lower Amazon River region. *Biochem Syst Ecol*. 2018;79:21-. doi:10.1016/j.bse.2018.04.017
- Tucker AO, Maciarelo MJ, Landrum LR. Volatile leaf oils of American Myrtaceae. II. *Calycolpus warszewiczianus* Berg of Costa Rica. *J Essent Oil Res*. 1993;5(5):561-562.
- Salehi B, Sharifi-Rad J, Quispe C, et al. Insights into *Eucalyptus* genus chemical constituents, biological activities and health-promoting effects. *Trends in Food Science & Technology*. 2019;91:609-624. doi:10.1016/j.tifs.2019.08.003
- Caldas GFR, Limeira MMF, Araújo AV, et al. Repeated-doses and reproductive toxicity studies of the monoterpene 1,8-cineole (eucalyptol) in Wistar rats. *Food Chem Toxicol*. 2016;97:297-306. doi:10.1016/j.fct.2016.09.020
- Martins AOBPB, Rodrigues LB, Cesário FRAS, et al. Anti-edematogenic and anti-inflammatory activity of the essential oil from *Croton rhamnifolioides* leaves and its major constituent 1,8-cineole (eucalyptol). *Biomed Pharmacother*. 2017;96:384-395. doi:10.1016/j.biopha.2017.10.005
- Kim H-M, Kwon H, Kim K, Lee S-E. Antifungal and Antiaflatoxinigenic Activities of 1,8-Cineole and t-Cinnamaldehyde on *Aspergillus flavus*. *Appl Sci*. 2018;8(9):1655. doi:10.3390/app8091655
- De Vincenzi M, Silano M, De Vincenzi A, Maialetti F, Scazzocchio B. Constituents of aromatic plants: eucalyptol. *Fitoterapia*. 2002;73(3):269-275. doi:10.1016/S0367-326X(02)00062-X
- Gottlieb O, Magalhães M. Modified distillation trap. *Chem Anal*. 1960;49(4):114
- Maia JGS, Andrade EHA. Database of the Amazon aromatic plants and their essential oils. *Quím. Nova*. 2009;32(3):595-622. doi:10.1590/S0100-40422009000300006
- van Den Dool H, Dec. Kratz P. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. *J Chromatogr A*. 1963;11:463-471. doi:10.1016/S0021-9673(01)80947-X