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Original Article

Chemical composition and seasonality variability of the *Spiranthera odoratissima* volatile oils leaves

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ABSTRACT

Spiranthera odoratissima A. St.-Hil., Rutaceae, known as "manacá" is a shrub native of the Brazilian Cerrado. Their leaves and roots are popularly used to treat rheumatism, infection and abdominal pain. This study analyzed the chemical composition of volatile oils from leaves of *S. odoratissima* and verified the seasonal variability of its chemical composition. The volatile oils were obtained by hydrodistillation using a Clevenger type apparatus and analyzed by gas chromatography coupled to mass spectrometry. The main chemical components found in samples of volatile oils were β-caryophyllene, bicyclogermacrene, δ-cadinene, amorphous-4,7(11)-diene, α-epi-muurolol, α-cadinol, α-muurolol and γ-cadinene. The hierarchical clustering identified three groups: the first was characterized by α-epi-muurolol, the second by amorphous-4,7(11)-diene and the third group was characterized by α-muurolol. The discriminant canonical analysis was used to differentiate between clusters on the basis of oil composition. The results suggest that the rainfall presented a relationship with the chemical composition of the volatile oil. This is the first study conducted on the seasonal behavior of the chemical constituents in volatile oil from leaves of *S. odoratissima*.

Introduction

Spiranthera odoratissima A. St.-Hil., Rutaceae, is a shrub known as "manacá" with erect stems that come together to form clumps very aromatic. It is native to Central Brazil (Cerrado), occurring mainly in Goiás, Mato Grosso and Bahia states. The leaves and roots are popularly used for treatment of various diseases such as syphilis, rheumatism, renal infections and urinary retention, abdominal pain, gout, furuncles and acne (Matos et al., 2003, 2014; Albernaz et al., 2010; Barbosa et al., 2012). *Spiranthera* sp. volatile oils and their components possess anti-inflammatory, analgesic (Silva et al., 2010; Matos et al., 2004, 2014), anxiolytic (Galdino et al., 2012) and antiprotozoal activities (Albernaz et al., 2012).

The chemical composition of secondary metabolites could be related to climate and atmospheric parameters. The temperature and precipitation were identified as factors that might influence the chemical composition of volatile oil (Cruz et al., 2014). Studies showed that the chemical patterns of the Cerrado species are directly related to the seasonality (Sá et al., 2016).

Water availability in the Cerrado defines two seasons: dry (April to September) and wet (October to March) (Santos et al., 2006). Thus, it is assumed that the secondary metabolism responds in two ways, depending on environmental conditions (Gobbo-Neto and Lopes, 2007; Oliveira et al., 2012; Cruz et al., 2014; Amaral et al., 2015).

The objective of this study was to analyze the chemical composition and seasonal variability volatile oil leaves of *S. odoratissima* over a seasonal cycle from November 2014 to October 2015.

Materials and methods

Plant material

Leaves of about fifty individuals of *Spiranthera odoratissima* A. St.-Hil., Rutaceae, were collected at 8 and 9 am, monthly, in the city of Aparecida de Goiânia, Goiás State,

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Brasil (16°45′45.2″ S/49°07′06.8″ W, 762 m), in the period between November 2014 to October 2015. Plant material was identified by Prof. Dr. José Realino de Paula and a voucher specimen was deposited at the Herbarium of the Federal University of Goiás, Brazil, under code UFG 60010. The leaves were dried at room temperature. Meteorological data of Aparecida de Goiânia, GO (November 2014 to October 2015) were obtained from the online climate database of the National Institute of Meteorology (INMET, 2013). For data analysis, monthly averages of temperature and accumulated rainfall and average daylength for each month were used.

Volatile oils extraction and GC–MS analysis

For the extraction of the volatile oil, leaves (115 g) were dried at room temperature for three days, triturated using commercial crusher (Skymsen, LS-08MB-N) immediately prior to the extraction of the volatile oil, avoiding loss by volatilization, and submitted to hydrodistillation in a Clevenger-type apparatus for 3 h. After dried over anhydrous Na_2SO_4, oils were stored in sealed brown vials and at −18 °C. The volatile oil volume was measured in the graduated tube of the apparatus and was calculated as percentage relative to the initial amount of dry plant material used in the extraction. Each experiment was performed in triplicate.

The volatile oil were analyzed using a gas chromatography coupled to mass spectrometry Shimadzu GC-MSQP5050A fitted with a fused silica SBP-5 (30 m × 0.25 mm I.D.; 0.25 m film thickness) capillary column (composed of 5% phenyl-methylpolysiloxane) and temperature programmed as follow: 60–240 °C at 3 °C/min, then to 280 °C at 10 °C/min, ending with 10 min at 280 °C. The carrier gas was a flow rate of 1 ml/min and the split mode had a ratio of 1:20. The injection port was set at 225 °C. Significant quadrupole mass spectrometer operating parameters: interface temperature 240 °C; electron impact ionization at 70 eV with scan mass range of 40–350 *m*/*z* at a sampling rate of 1 scan/s. Constituents were identified by computer search using digital libraries of mass spectral data (NIST, 1998) and also by comparison of their retention indices (Van Den Dool and Kratz, 1963) relative to C_8–C_{32} *n*-alkanes and mass spectra with literature data (Adams, 2007).

Statistical analysis

The Principal Component Analysis (PCA) was applied to analyze the interrelationships between the chemical constituents of leaves of volatile oil collected in different months using Statistica 7.0 software (StatSoft Inc., Oklahoma, USA). The hierarchical cluster analysis (HCA) was used to study the similarity between the samples according to the distribution of the constituents, and this analysis was performed by Ward's method (Ward, 1963). To validate the cluster analysis was performed the canonical discriminant analysis (CDA). The predictive ability of linear discriminant functions was evaluated by cross-validation. The *p* values less than 0.01 were considered significant. Prior to the multivariate analysis, the data were preprocessed by means of auto-scaling and mean centering. The Pearson's correlation between β-caryophyllene and daylength average was performed to verify their possible association.

Results

Climate data collection of plant material period of *S. odoratissima* is described in Table 1. In November 2014 to April 2015, there was high rainfall with values ranging from 170.5 to 337.9 mm, respectively, except for January 2015 that had an atypical behavior recording a value of 73.6 mm. The months of June 2015 July 2015 and August 2015 is presented as the months of extreme drought, reaching the highest rainfall value of 3.6 mm in August, September 2015 (30.4 mm) and October 2015 (18.2 mm). May stands out with an intermediate rainfall value of 70.7 mm. The same behavior is observed for the relative humidity (%), higher humidity during the rainy season (March 2015) and lower in the dry season (August 2015), with values of 75.9 and 38.4%, respectively.

Table 1
Climatic information of the period of collection of plant material of *Spiranthera odoratissima*.

Date	Rainfall precipitation (mm)	Relative humidity (%)	Daylight (h)	Average temperature (°C)	
				Maximum	Minimum
11/30/2014	170.5	67.3	163.5	31.9	20.8
12/31/2014	337.9	72.4	144.1	30.2	20.2
01/31/2015	73.6	56.5	249.9	34.0	21.0
02/28/2015	225.2	70.2	165.7	31.0	20.4
03/31/2015	312.3	75.9	148.5	30.0	20.1
04/30/2015	204.2	72.5	188.2	31.0	20.6
05/31/2015	70.7	66.0	225.2	29.7	18.4
06/30/2015	0.0	56.3	250.8	30.2	17.0
07/31/2015	2.7	50.9	252.5	31.4	17.0
08/31/2015	3.6	38.4	283.7	33.1	17.4
09/30/2015	30.4	42.5	251.0	36.0	20.4
10/31/2015	18.2	43.6	242.5	36.8	22.2

Source: INMET (Goiânia Station – OMM: 83423). 2013.

Temperature variations during the collection period were not significant, with temperatures ranging from 17 °C to 36.8 °C, respectively (Table 1).

Volatile oil

The volatile oils yield ranges from 2.3% in November (rainy) to 3.4% in July (low rainfall).

Through the GC–MS analysis identified 41 chemical compounds. The highest percentages of identified chemical compounds occurred in the rainy months, especially the month of March 2015 to 99.42% while the lowest percentages identified, occurred in the dry season, reaching a minimum of 81.13% in July 2015 (Table 2). The class of sesquiterpene hydrocarbons showed more highlight with 26 compounds (from 67.95 to 86.17%), with higher values during the rainy season, followed by oxygenated sesquiterpenes (12 compounds) in small percentages (5.73–19.00%) and a minority of monoterpenes hydrocarbons with quantities below 2%.

The major chemical components of the samples along the seasonal cycle were β-caryophyllene (6.78–12.15%), bicyclogermacrene (17.61–23.08%), δ-cadinene (12.31–16.55%) and amorphous-4,7(11)-diene (10.71–19.87%).

The major compounds α-epi-muurolol and α-cadinol were not identified in the June to August and June to October (dry months), respectively. These compounds were produced in similar amounts in December 2014, when the highest rainfall occurred in the period, with the lowest percentage of 4.34% (α-epi-muurolol) and 4.89% (α-cadinol), while the highest values were 6.25% (November 2014) and 8.35% (May 2015), respectively. α-Muurolol presented a slight percentage increase in the dry months of July (7.65%) and August (5.10%). The compound γ-cadinene was identified in all months with a percentage ranging of 2.37% (May/2015) and 5.68% (September 2015), respectively. It was verified that there was no correlation between beta caryophyllene and daylength ($R = 0.057$, $p = 0.86$).

The results obtained from the Principal Component Analysis (PCA) and Cluster analysis (CA) (Figs. 1 and 2) indicate large chemical variability in the samples of *S. odoratissima* EO. The majority of the data could be represented in two main axes, which contained 87.4% of total variance (PC1 = 62.5, and PC2 = 24.88%; Fig. 1). The two-dimensional representation of the first two axes of the PCA

Table 2
Percentage of the chemical constituents of volatile oils from leaves of *Spiranthera odoratissima* in the annual cycle from November 2014 to October 2015.

Constituents	KI	2014		2015									
		Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	July	Aug	Sep	Oct
α-Pinene	939	0.22	0.42	0.86	0.60	0.78	0.66	0.60	0.33	1.23	0.24	–	0.29
Myrcene	990	–	–	0.10	–	–	–	–	–	0.19	–	–	–
Limonene	1029	0.35	0.42	0.6	0.48	0.56	0.56	0.68	0.31	0.57	0.25	–	0.24
α-Cubebene	1348	0.77	0.93	0.35	0.58	0.70	0.44	0.42	0.80	0.66	0.35	0.64	0.31
α-Copaene	1376	3.27	3.70	2.76	2.86	2.86	2.74	2.74	3.19	–	3.12	3.07	2.43
β-Bourbonene	1388	–	0.17	–	–	–	–	–	–	–	–	–	–
β-Cubebene	1388	0.64	0.79	0.48	–	–	–	–	–	–	–	–	–
iso-Longifolene	1389	–	–	–	0.63	0.67	0.66	0.61	0.79	0.90	0.71	0.67	0.49
β-Elemene	1390	1.32	1.44	1.38	1.37	0.82	0.92	0.82	0.59	0.74	1.17	0.63	0.96
β-Caryophyllene	1419	9.83	12.01	9.32	6.78	10.86	9.94	10.89	12.04	8.15	9.74	12.15	11.04
β-Copaene	1432	0.32	0.44	0.28	0.13	–	0.78	–	0.31	–	–	0.53	–
α-*trans*-Bergamotene	1434	–	0.18	0.18	–	–	–	–	0.43	–	0.14	–	–
Aromadendrene	1441	0.74	0.97	0.63	0.62	–	–	0.26	0.54	0.21	0.32	0.57	0.51
cis-Muurola-3,5-diene	1450	–	–	0.20	0.18	–	–	0.24	0.33	0.17	–	0.59	0.25
trans-Muurola-3,5-diene	1453	–	–	–	0.33	–	–	–	–	–	0.30	0.45	0.39
α-Humulene	1454	2.61	2.86	2.63	2.34	2.27	2.56	2.47	2.51	2.22	2.89	2.66	2.68
allo-Aromadendrene	1460	3.07	3.32	3.36	3.02	2.76	2.90	2.70	2.80	2.86	3.16	3.35	2.77
trans-Cadina-1(6),4-diene	1476	0.31	–	0.55	0.48	–	0.48	0.46	0.61	0.38	1.31	0.77	0.62
γ-Muurolene	1479	1.56	1.82	1.46	1.40	0.95	1.13	1.03	1.42	0.99	–	1.62	1.27
Amorphous -4,7(11)-diene	1481	11.46	13.27	10.71	16.35	19.87	15.67	16.3	17.22	11.75	15.00	15.33	13.32
β-Selinene	1490	0.52	0.39	0.44	0.42	–	–	–	0.41	–	0.42	0.67	0.44
trans-Muurola-4(14),5-diene	1493	0.62	0.57	0.88	0.77	–	1.00	0.87	0.84	0.85	0.64	0.92	0.80
Bicyclogermacrene	1500	20.03	19.75	22.35	23.08	20.89	21.11	20.12	21.34	18.48	19.40	17.61	18.88
α-Muurolene	1500	–	–	–	–	2.02	2.43	1.99	–	–	–	–	–
Germacrene A	1509	1.39	1.11	1.51	1.60	1.16	1.72	1.58	1.23	1.41	1.98	1.25	1.56
γ-Cadinene	1513	4.60	4.72	4.60	3.61	2.79	2.64	2.37	3.35	2.58	2.94	5.68	3.18
δ-Cadinene	1523	15.08	12.31	16.55	15.83	14.49	15.67	14.01	14.38	14.70	14.90	13.67	15.87
trans-Cadina-1,4-diene	1534	0.26	–	0.42	0.41	–	0.42	0.39	0.45	0.37	–	0.49	0.40
α-Cadinene	1538	0.81	0.85	0.91	0.74	0.53	0.63	0.56	0.59	0.53	0.63	0.83	0.63
Germacrene D-4-0l	1575	–	–	–	–	–	–	0.68	–	–	–	–	–
Espathulenol	1578	2.36	3.30	0.85	0.92	–	–	–	–	–	–	–	–
Caryophyllene oxide	1583	–	1.08	–	–	–	–	–	–	–	–	–	–
Globulol	1590	0.92	0.52	0.6	0.61	–	0.41	0.57	–	–	–	1.88	–
β-Copaen-4-α-ol	1590	–	0.24	–	0.43	–	–	–	0.50	2.12	2.30	–	0.58
Guaiol	1600	–	–	–	–	–	–	–	0.20	0.66	0.21	0.24	0.17
Ledol	1602	–	0.27	0.57	0.22	–	–	–	–	–	–	–	–
1,10-di-*epi*-Cubenol	1619	0.23	0.22	0.20	0.17	–	–	–	0.16	0.17	0.22	0.61	0.17
1-*epi*-Cubenol	1628	0.69	0.51	0.51	0.50	–	0.43	0.47	0.47	0.59	0.48	0.25	0.50
α-*epi*-Muurolol	1642	6.25	4.34	5.39	5.01	5.95	5.39	6.04	–	–	–	4.35	5.76
α-muurolol	1646	1.00	0.86	0.81	0.73	0.83	0.75	0.90	4.40	7.65	5.10	0.55	–
α-Cadinol	1654	7.55	4.89	5.93	5.83	7.66	6.72	8.35	–	–	–	–	–
Monoterpene hydrocarbons		0.57	0.84	1.56	1.08	1.34	1.22	1.28	0.64	1.99	0.49	0.00	0.53
Sesquiterpene hydrocarbons		79.21	81.6	81.95	83.53	83.64	83.84	80.83	86.17	67.95	79.12	84.15	78.8
Oxygenated sesquiterpenes		19.00	16.23	14.86	14.42	14.44	13.70	17.01	5.73	11.19	8.31	7.88	7.18
Total identified		98.78	98.67	98.37	99.03	99.42	98.76	99.12	92.54	81.13	87.92	92.03	86.51
Yield (%)		2.3	2.5	2.3	2.6	2.8	2.6	2.6	2.6	3.4	3.1	3.0	3.1

–, absent; KI, Kovats retention index.

can be visualized in Fig. 1. Through the PCA, it was verified that the variables selected in the 12 samples can be represented by two main components (CP) that account for most of the system variance. Canonical discriminant analysis (CDA) was performed to help predict the three groups using only two predictive variables: amorphous-4,7(11)-diene and α-muurolol (Table 3). The results showed that the discriminant function retain 100% of well-classification in the original clusters ($p < 0.01$).

Discussion

The climate data of Aparecida de Goiânia, GO presented two well-defined seasons, one rainy and one dry. In the dry period, yields of *S. odoratissima* volatile oils were higher, reaching a maximum of 3.4% in July. Chaibub et al. (2013) verified a yield of 2.3% in the volatile oil of the leaves of *S. odoratissima* collected in December in Senador Canedo, GO. Verma et al. (2014) also found differences in volatile oil yields of *Aegle marmelos* (L.) Correa (Rutaceae), collected in India, in the different seasons of the year, with values of 0.37–0.82%. Santos et al. (2016) observed an increase in volatile oil yield of the leaves of *Hortia oreadica* in the rainy season.

In the present study, there was a predominance of sesquiterpene hydrocarbons (67.95–86.17%). Iñigo et al. (2002) observed a predominance of sesquiterpene hydrocarbons in leaves of *Haplophyllum linifolium* (L.) G. Don fil. (Rutaceae). The majority components were bicyclogermacrene (9.0%) and β-caryophyllene (7.5%). On the other hand, Sun et al. (2015) verified a predominance of monoterpenes in the leaves of *Dictamnus angustifolius* (Rutaceae) leaves.

The major chemical components of the *S. odoratissima* volatile oils with the highest amounts in the rainy months were bicyclogermacrene (23.08% in February), amorphous-4,7(11)-diene (19.87% in March), δ-cadinene (16.55% in January) and β-caryophyllene (12.15% in September). Three clusters were identified through cluster and PCA analysis: cluster I corresponded to a period of the beginning of the rains, cluster II corresponded to a period of high rainfall and cluster III corresponded to the dry season and. In the DCA analysis, the combination of the compounds showed that

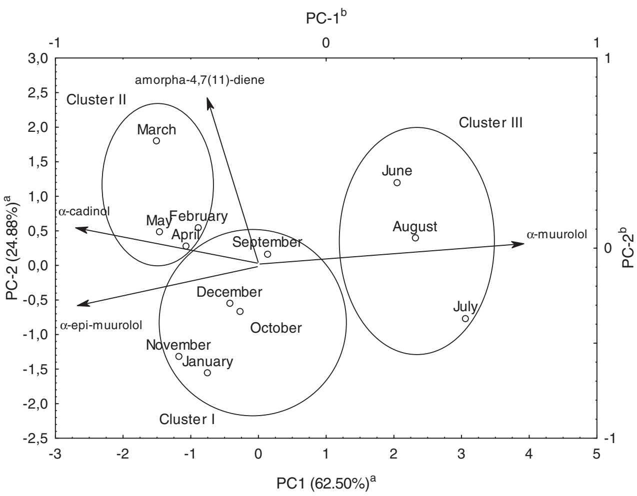

Fig. 1. PCA scatter plot of three clusters obtained by the sequential HCA from the PCA scores of the chemical components of the volatile oils from leaves of *Spiranthera odoratissima*. [a]Axial for the sample scores. [b]Axial relation to the discriminant scores of the chemical constituents of volatile oils represented by the origin vectors.

Table 3
Canonical discriminant analysis summary of *Spiranthera odoratissima*.

	Canonical discriminant			
	Eingenvalues functions	Canonical *R*	Wilk's Lambda	*p*-Level
F1	16.01	0.97	0.03	0.000005
F2	0.95	0.69	0.51	0.016951
		Standardized coefficients		
Amorphous-4,7(11)-diene		−0.9195		−0.9433
α-Muurolol		−1.3147		0.0838
Eigenvalues		16.01		0.95
Cumulative proportion		0.94		1.00
		Percentage of total well-classification		
Cluster I		100		$p = 0.42$
Cluster II		100		$p = 0.33$
Cluster III		100		$p = 0.25$

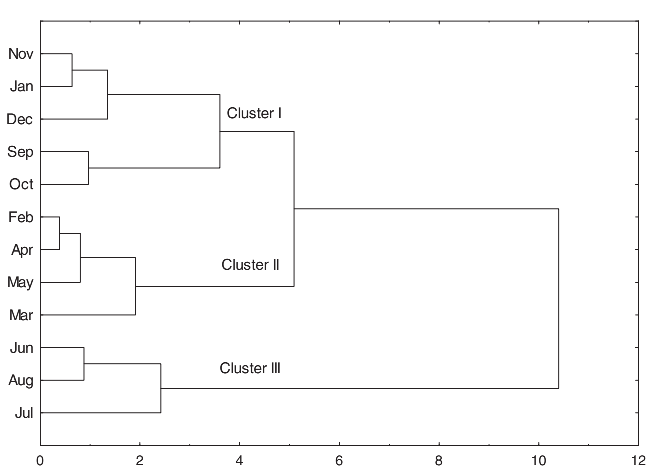

Fig. 2. Dendrogram of similarity based on the Euclidian distance, in relation to the collection period, from the PCA scores with three groupings of the volatile oils chemical compounds from leaves of *Spiranthera odoratissima*.

amorphous-4,7(11)-diene and α-muurolol were the predicted variables suitable for correct classification of all samples from the data set, validating the analysis of HCA ($p \leq 0.01$) with a correct classification percentage of 100%. It was verified that there was no correlation between β-caryophyllene and daylength. Of the environmental factors, temperature, season of the year, water stress and time of exposure to light, the incidence of light directly influences the synthesis of chemical substances in some species depending on the family. According Simões and Spitzer (2010) the seasonal contrasts are evidenced in families of plants that have histological structures of storage of volatile oil on the surface of the plant (glands), is not the case of Rutaceae that store volatile oil in the secretory cavities. In the literature there are several reports about the influence of seasonality on the chemical composition of volatile oils of Rutaceae species, however, there are no studies for *Spiranthera*. In *Hortia oreadica* Groppo, Kallunki & Pirani, Rutaceae, the bicyclogermacrene was found to be the major component, with the highest percentage in the rainy period (31.37%, November), while the amorphous-4,7(11)-diene chemical component was present, in higher percentages, in the dry period (37.89%, August) (Santos et al., 2016).

Chaibub et al. (2013) found as major compounds of *S. odoratissima* volatile oils, the β-caryophyllene (20.64%) bicyclogermacrene (14.73%) and δ-cadinene (13.40%). Christofoli et al. (2015) found as main components in the volatile oil of *Zanthoxylum rhoifolium*, Rutaceae, leaves the β-caryophyllene (12.09%) and bicyclogermacrene (4.57%). β-Caryophyllene was also found in other species of the Rutaceae, as *Zanthoxylum syncarpum* Tull. (9.35%) (Nunes, 2009), *Aegle marmelos* (L.) Correa (5.30%) (Verma et al., 2014), *Zanthoxylum avicennae* (Lam.) DC. (5.09%) (Liu et al., 2014), *Murraya exotica* (L.) (7.05%) (Krishnamoorthy et al., 2015) and six species of the genus *Murraya*, Rutaceae, *M. tetramera Huang* (8.15%), *M. euchrestifolia* Hayata (12.94%), *M. koenigii* (L.) Spreng (27.73%), *M. kwangsiensis* (Huang) Huang (14.9%), *M. exotica* L. (20.29%) and *M. alata* Drake (17.44%) (You et al., 2015).

Bicyclogermacrene was also found in the volatile oil l of *Phebalium squamulosum* subsp. Coriaceum Paul G. Wilson leaves (Sadgrove et al., 2014). The δ-cadinene was not found, in the researched literature, in volatile oil of other species of Rutaceae, and can be used as a marker of *S. odoratissima*.

It can be concluded that the chemical variability and yield of *S. odoratissima* volatile oil were influenced by seasonality. Rainfall was the meteorological factor that most influenced the chemical composition of the volatile oils of *S. odoratissima* leaves. The major constituents of volatile oils were β-caryophyllene, δ-cadinene, amorphous-4,7(11)-diene, bicyclogermacrene. The knowledge acquired from this study becomes important for further exploration of the volatile oil of this species.

Authors' contributions

SJOS contributed in collecting plant sample, running the laboratory work and drafted the paper. PHF contributed to chromatographic analysis. TSF contributed to critical reading of the manuscript. LLB contributed to the statistical analyzes. JRP designed the study, supervised the laboratory work, contributed to biological and chemical studies, chromatographic analysis and contributed to critical reading of the manuscript. All the authors had read the final manuscript and approved the submission contributed to biological studies running the laboratory work, analysis of the data.

Conflicts of interest

The authors declare no conflicts of interest.

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