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Artigo

SEASONAL VARIATION OF THE MAJOR SECONDARY METABOLITES PRESENT IN THE EXTRACT OF *Eremanthus mattogrossensis* Less (Asteraceae: Vernonieae) LEAVES[#]

Dayana Rubio Gouvea, Leonardo Gobbo-Neto, Humberto T. Sakamoto, Norberto Peporine Lopes e João Luís Callegari Lopes*
Departamento de Física e Química, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Av. do Café, s/n, 14040-903 Ribeirão Preto – SP, Brasil
Fernando Meloni
Departamento de Biologia, Faculdade de Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Av. Bandeirantes, 3900, 14040-901 Ribeirão Preto – SP, Brasil
Juliano Geraldo Amaral
Instituto Multidisciplinar em Saúde, Universidade Federal da Bahia, Rua Rio de Contas, 58, Quadra 17, Lote 58, 45029-094 Vitória da Conquista – BA, Brasil

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The species *Eremanthus mattogrossensis*, known as "veludo do cerrado" (cerrado velvet), is native to the Brazilian Cerrado. Because the amount of metabolites present in plants may be influenced by biological and environmental factors, here we conducted an HPLC-DAD-MS/MS investigation of the metabolite concentrations found in the MeOH/H_2O extract of the leaves of this species. The main compounds were identified and quantified, and the metabolites were grouped by chemical class (caffeoylquinic acids, flavonoids, and sesquiterpene lactone). Statistical analysis indicated a straight correlation between the quantity of metabolites and seasonality, suggesting that environmental properties elicit important metabolic responses.

Keywords: *Eremanthus mattogrossensis*; HPLC-DAD-MS/MS methodology; seasonal variation.

INTRODUCTION

Despite the existence of genetic control, gene expression, and genotypes, the total content and relative proportions of secondary metabolites in plants may vary over time and space (seasonal and daily variation as well as intraplant, interplant, and interspecies distinctions), so that they occur at different levels.[1-3] The amount of metabolites present in a given plant may be influenced by biological and environmental factors[4] as well as biochemical, physiological, ecological, and evolutionary processes.[3,5] Seasonality, circadian rhythm, plant development, phenology, temperature, altitude, water availability, UV radiation, nutrients, pollution, mechanical stimuli, and attacks by herbivores or pathogens are considered to be the factors that most affect the occurrence of plant metabolites.[4,6]

Most of the studies about the factors influencing secondary metabolite concentration are restricted to a few commercially important species native mainly to temperate regions. These species have long been manipulated by men, and the cultivated specimens have stood selective pressure due to choice of samples with the characteristics desired by humans. Because these plants may have lost their original behavior, their wild behavior cannot be inferred from the currently cultivated specimens.[1,7]

Specific *in situ* and *in locus* studies are mandatory for better understanding of the chemical behavior and more thorough appreciation of the variation in metabolite concentrations of wild plants in tropical ecosystems. This information is necessary for both evolutionary and chemotaxonomic studies and shall assist expansion of the current knowledge about the ecological interactions taking place between a certain plant and its surroundings.[8]

Cerrado is the second largest Brazilian biome. It stands out due to its wide variety of animals and plant species, which in turn is associated with a large array of environments. The 22 species of the genus *Eremanthus* Less (Asteraceae: Vernonieae) are restricted to this biome. The species *Eremanthus mattogrossensis* Less. is widely distributed in the western area of the Central Brazilian Plateau and exists at elevations ranging from 500 to 1,000 m in the Cerrado. This plant is particularly common in Mato Grosso and is popularly known as "veludo do cerrado" (cerrado velvet) and "casca freta" (cleft bark). Phytochemical analysis of the genus *Eremanthus* has revealed the presence of flavonoids, polyacetylenes, triterpenes, and sesquiterpenes, and sesquiterpene lactones have been very often reported as secondary metabolites in this plant.[9] In this work, we aimed to identify and quantify the major compounds found in *E. mattogrossensis* and to evaluate the seasonal variation in their concentrations.

EXPERIMENTAL

General experimental procedures

Chemicals

HPLC grade methanol (MeOH), acetonitrile (MeCN), and acetic acid were obtained from J.T. Baker. De-ionized water 18 mΩ (Milli-Q, Millipore) was employed in all the experiments.

Equipment

HPLC analysis was conducted on a Shimadzu HPLC-DAD, LC-6AD pump coupled to a Diode Array Detector (SPD-M10Avp, Shimadzu), and to an auto injector (SIL-10AF, Shimadzu), all controlled by the software CLASS-VP 6.14. A LC-RP-18 column (5 μm, 4.6/250 mm; Sigma-Aldrich) connected with a guard-column (4.6/10.0 mm) composed of equivalent material was utilized.

*e-mail: joaoluis@usp.br
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Plant material

E. mattogrossensis branches were collected in the municipality of Delfinópolis, state of Minas Gerais, Brazil (S 20°20'55.0', W 046°47'63.8''; 864 m altitude) and identified by Prof. Dr. J. Semir of the Botany Department of the Biology Institute of the University of Campinas (UNICAMP), state of São Paulo, Brazil. A voucher specimen was deposited under the code NPL 288 in the Herbarium of this same institution.

As soon as possible, the plant material was dried under forced ventilation at 40 °C, for 48 h, and stored in a dry place, away from light and insect attack.

Ten specimens were collected from the same population on the same day and at the same time. Three specimens were collected on different days, at one-month intervals, for 21 months (Jun/2000-Feb/2002), at 12:00 pm (± 30 min), for the seasonal studies.

Sample preparation for chromatographic analysis

The leaves of each *E. mattogrossensis* branch were ground in an analytical mill. The powdered leaves (30 mg) were weighed in a glass vial, to which 3 mL of a MeOH/H_2O (9:1) solution containing the internal standard coumarin (30 μg mL^{-1}) were added. This mixture was subjected to extraction in ultrasonic bath for 10 min. The resulting extract (500 μL) was transferred to a centrifuge tube (1.5 mL), to which 500 μL hexane were added. The tube was vortex-stirred and centrifuged at 1200 g for 10 min. A 300 μL aliquot was removed from the hydro-alcoholic phase and filtered on a 0.45-μm cellulose acetate membrane. The filtrate (20 μL) was submitted to HPLC analysis.

Analytical HPLC method

The following mobile phase gradient was employed for HPLC-DAD analysis, at a flow rate of 1.6 mL min^{-1}: solvent A = aqueous acetic acid, 2% (v/v); solvent B = MeCN, 98%-acetic acid, 2% (v/v); elution profile = 0-35 min, 10-18% B (linear gradient), 35-40 min, 18-23% B (linear gradient), 40-50 min, 23-28% B (linear gradient), 50-60 min, 28-40% B (linear gradient), 60-65 min, 40-100% B (linear gradient), 65-70 min (column washing), 100% B (isocratic), 70-75 min, 100-10% B (linear gradient), 75-80 min (column equilibration), 10% B (isocratic); the UV-DAD detector was set to record spectra between 210 and 600 nm, and UV chromatograms were recorded at 270 and 325 nm.

HPLC-DAD-MS and –MS/MS analyses

HPLC-DAD-MS and –MS/MS analyses were performed by using the same column and elution gradient described for the analytical HPLC method. The parameters and apparatus used in the mass spectrometry analysis were the same as those described in a previous study published by our research group.[10]

Method validation

The analytical methodology was validated in accordance with current Brazilian and international legislations,[11] using the same parameters employed for the previously described methodology,[10] except for the fact that at least one substance of each class of secondary metabolite identified in the *E. mattogrossensis* leaf extracts was utilized. On the basis of availability, 4 compounds were chosen for construction of the analytical curves and method validation, namely di-*C*-glucosylflavone vicenin-2 (6,8-di-*C*-βglucopyranosylapigenin), chlorogenic acid (5-*O*-*E*-caffeoylquinic acid), 3,4-di-*O*-*E*-caffeoylquinic acid, and the sesquiterpene lactone goyazensolide. Peak areas were calculated at 325 nm for flavonoids and caffeoylquinic acids, and at 270 nm for the sesquiterpene lactone goyazensolide.

The internal standard (I.S.) method was utilized for construction of analytical curves for the standards of these compounds at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0, 200.0, 500.0, and 1000.0 μg mL^{-1}, in triplicate. The overall recovery level of the method was determined by spiking 50.0, 100.0, and 150.0 μg of each standard substance into a matrix consisting of dried and powdered *E. mattogrossensis* leaves (30.0 mg) that had been previously exhaustively extracted (5 times) by the same extraction method described above and then dried.

Statistical analysis

Exploratory multivariate techniques were employed for assessment of the seasonality effect on the quantified metabolites. The climate variables maximum temperature, minimum temperature, and rainfall were selected for identification of the natural seasonal groups. Cluster analysis performed by the hierarchical method adopting Euclidian distance as similarity measure and Ward method for linkage groups was utilized. In the next step, the non-hierarchical method K-means was used for detection of variables within the groups. The groups identified by the hierarchical method and K-means were tested by Hotelling's T^2 test,[12] to confirm the differences between groups.

To evaluate the variation of metabolite concentrations, the metabolites were grouped by chemical class (caffeoylquinic acids, flavonoids, and sesquiterpene lactone), and the metabolites concentration values were added up according to the respective chemical class. The values resulting from each metabolite class were categorized into 4 concentration levels (a, b, c, d), which contained a similar number of samples (each category had around 25% of the total number of samples). The samples were grouped according to the month/season, in accordance to the cluster analysis data. Multivariate correspondence analysis (CA) was applied for better understanding of the effect of seasonal variation on the amount of each class of chemical compound. The relationship amount variation/chemical classes/seasons was examined by correlation with the dimensional axis calculated by CA.[13] The software Statistica 7.0 was employed for all the data analysis.

RESULTS AND DISCUSSION

Chromatographic peak identification

The chromatographic signals identification was conducted as previously published by our research group.[10] To this end, the chemosystematics information about the genus was compared to the UV spectra and the data generated by mass spectrometry. Peak identity was confirmed by co-injection of standard compounds previously isolated by our group, when possible. In the case of chlorogenic acid derivatives, they have characteristic fragmentation profiles in ESI previously described in the literature.[14] In this case, only the data from MS/MS, accurate mass, UV spectrum and elution order in reverse phase chromatography allow the correct identification of compounds.

The accurate mass obtained for all peaks were in agreement with the exact masses calculated for the deprotonated molecule relative to each compound (Table 1). Only the identified signals are described below. Figure 1 illustrates an example chromatogram and the peaks numbering, including the non-identified flavonoids (flavonoids NI: peaks 3, 5, 8 e 10). It is important to note that for this species have been reported two sesquiterpene lactones[15] and only one was identified at the present work (Figure 2).

Table 1. Accurate and exact calculated mass in negative ionization mode obtained for each identified compound present in the methanol/water extract of *Eremanthus mattogrossensis* leafs

peak	compound	Accurate mass $[M-H]^-$	Exact mass $[M-H]^-$	error (ppm)
1	3-*O*-*E*-caffeoylquinic acid	353,0872	353,0878	1,7
2	5-*O*-*E*-caffeoylquinic acid	353,0858	353,0878	5,7
4	Vicenin-2	593,1529	593,1511	3,0
6	3,4-di-*O*-*E*-caffeoylquinic acid	515,1185	515,1195	1,9
7	3,5-di-*O*-*E*-caffeoylquinic acid	515,1213	515,1195	3,5
9	4,5-di-*O*-*E*-caffeoylquinic acid	515,1226	515,1195	6,0
11	Goyazensolide	359,1117	359,1136	5,3

3-O-E-caffeoylquinic acid

The first isomer identified by mass spectrometry in the negative ionization mode was the ion *m/z* 353, which was attributed to the deprotonated molecule characteristic of mono-caffeoylquinic isomers. Fragmentation of this ion generated the ion *m/z* 191 as the base peak and the ion *m/z* 179 as the secondary ion in the MS2 spectra. These data allowed us to assign this substance (signal number 1) as 3-*O*-*E*-caffeoylquinic acid.

5-O-E-caffeoylquinic acid

The chromatographic signal number 2 displayed the ion *m/z* 353 (deprotonated molecule) and a fragment ion *m/z* 191 in the mass spectrum (negative ionization mode). Its UV spectrum was typical of caffeoylquinic acid (UV max: ~ 299 and 325 nm). Taken together, these data suggested quinic acid esterified with a single unit of caffeic acid. Comparison of the retention time of this substance with a standard confirmed this signal as 5-*O*-*E*-caffeoylquinic acid.

3,4-di-O-E-caffeoylquinic acid

Signal number 6 also exhibited the characteristic UV spectrum of caffeoylquinic acid described above. The base peak in the mass spectrum recorded in the negative ionization mode was the ion *m/z* 515, which indicated the presence of a positional isomer of di-caffeoylquinic acid. The peak obtained in the positive ionization mode was the ion *m/z* 499, consistent with a dehydrated di-caffeoylquinic acid $[M+H - H_2O]^+$. However, $[M+H]^+$ 517 and $[M+Na]^+$ 539 were also detected. Again, key data on the identification of caffeoylquinic acids by HPLC-DAD-MS/MS,[14] the presence of the ions *m/z* 191, 179 and 173 in the mass spectrum obtained from the fragmentation of the deprotonated molecule *m/z* 515, and comparison of the retention time with that of a standard allowed identification of this peak as 3,4-di-*O*-*E*-caffeoylquinic acid.

3,5-di-O-E-caffeoylquinic acid

The signal designated number 7 also presented the characteristics of di-caffeoylquinic acid. However, considering the presence of the ions *m/z* 191 and *m*/z 179 in the mass spectrum obtained from fragmentation of the ion *m/z* 515 in the negative ionization mode, and taking the order of elution into account, this signal was identified as 3,5-di-*O*-*E*-caffeoylquinic acid.

4,5-di-O-E-caffeoylquinic acid

For identification of this compound (signal designated number 9) the absence of the ion *m/z* 191 and the presence of the ions *m*/z 179 and *m*/z 173 in the mass spectrum obtained from fragmentation of the ion *m/z* 515 in the negative ionization mode were considered, together with comparison of the retention time with a standard.

Vicenin-2

The chromatographic signal labeled number 4 displayed UV spectrum with maximum absorption around 270 and 330 nm. The compound also produced fragmentation ions typical of

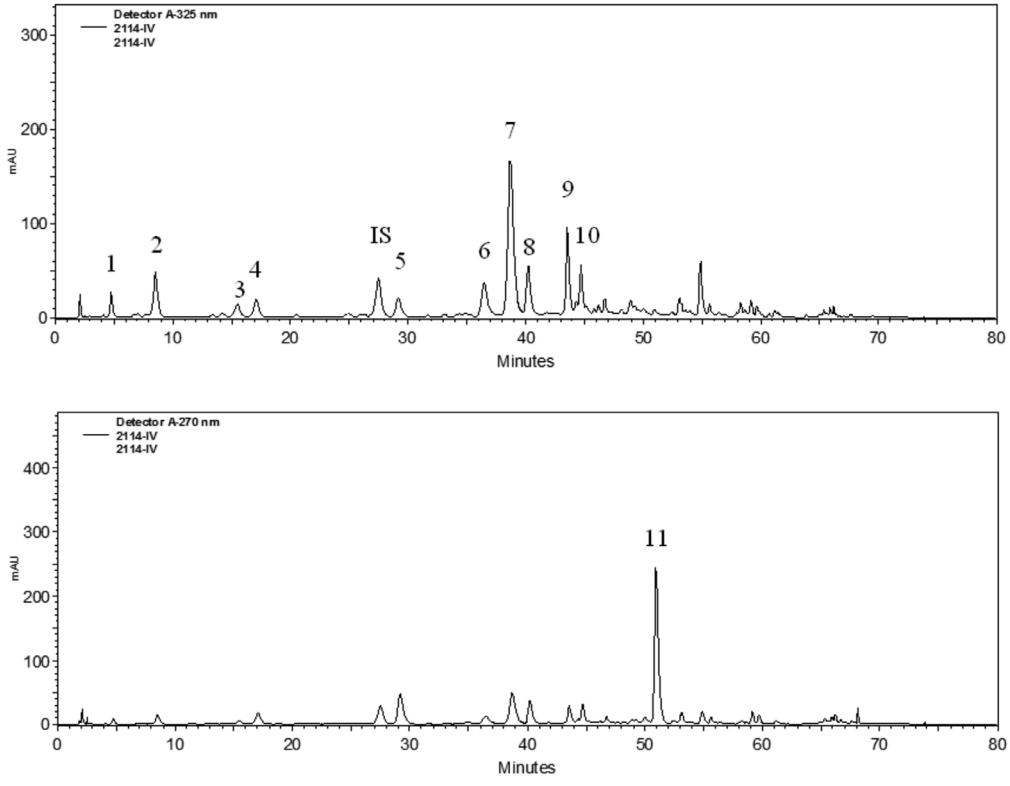


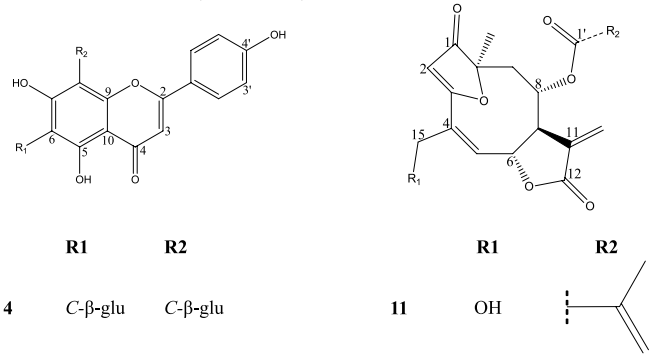
Figure 1. *Chromatograms (325 and 270 nm) obtained during analysis of the methanol/water extract obtained from ground leaves of Eremanthus mattogrossensis*

di-*C*-glucosylflavones, such as those generated from water loss and sugar fragmentation. Fragmentation of the ion 595 $[M+H]^+$ was verified in the positive ionization mode, as evidenced by the peaks at 577 $[M+H - 18]^+$, relative to water loss, and 457 $[M+H - 120]^+$, corresponding to loss of sugar parts. Comparison of the retention time of this signal with a standard enabled its identification as vicenin-2.

Goyazensolide

Identification of signal number 11 was accomplished by comparison with the retention time (co-elution) of a previously isolated authentic standard. The accurate mass obtained for this peak was in agreement with the exact masses calculated for the deprotonated molecule of this compound, and the product ion spectrum exhibited the same previously described fragmentation patterns and diagnostic ions.[16]

	R1	R2	R3
1	caffeoyl	H	H
2	H	H	caffeoyl
6	caffeoyl	caffeoyl	OH
7	caffeoyl	H	caffeoyl
9	H	caffeoyl	caffeoyl



	R1	R2
4	*C*-β-glu	*C*-β-glu

	R1	R2
11	OH	

Figure 2. *Compounds identified in the methanol/water extract obtained from ground leaves of Eremanthus mattogrossensis*

Method validation

Analytical method validation ensured reliability and credibility of the results for the purposes of this study and covered the concentration range required for chemical variability analysis. The validated method presented suitable limits of detection and quantitation, as well as satisfactory precision and recovery. All the results are listed in Tables 2, 3 and 4.

Sensitivity, accuracy, and precision data obtained via validation of the analytical method by HPLC-DAD are summarized in Tables 3 and 4.

Table 2. Linear regression equations and correlation coefficients for the standards employed during method validation

Standard compound	Linear regression equation	Correlation coefficient
5-*O*-*E*-caffeoylquinic acid	y = 0.0455x + 0.0141	R^2 = 0.9999
3,4 di-*O*-*E*-caffeoylquinic acid	y = 0.0286x + 0.0102	R^2 = 0.9999
vicenin-2	y = 0.0149x + 0.0316	R^2 = 0.9995
goyazensolide	y = 0.0122x – 0.0256	R^2 = 0.9996

Statistical analysis

Figure 3 reveals that the caffeoylquinic acids are more intense as compared to the concentration of all the chemical compounds belonging to other classes, in all the studied periods. The lowest flavonoids and caffeoylquinic acids concentrations were verified in March (0.51 μg mL^{-1} and close to 4.78 μg mL^{-1}, respectively). Sesquiterpene lactone was also present in low quantity in this period. The highest mean caffeoylquinic acids concentration values were detected in December (20.05 μg mL^{-1}) and February (22.66 μg mL^{-1}). As for flavonoids, the major mean values occurred in November (5.7 μg mL^{-1}) and December (6.63 μg mL^{-1}). Concerning sesquiterpene lactone, the largest mean values were found in October (6.49 μg mL^{-1}) and December (5.91 μg mL^{-1}), whereas the lowest mean value was registered in July (3.62 μg mL^{-1}).

Within the caffeoylquinic acids and flavonoids classes, there was no marked variation in metabolite proportions, and the respective metabolites presented equivalent means and standard error (Table 5). The compound 3,5-di-*O*-*E*-caffeoylquinic acid was the most abundant among the caffeoylquinic acids, comprising approximately 57.8% of the total, while 3-*O*-*E*-caffeoylquinic acid was present in the lowest proportion (4.5%). The other caffeoylquinic acid isomers represented between 10.3 and 14.7% of the total amount of caffeoylquinic acids. In the case of flavonoids, the non-identified metabolites numbered 3 and 5 were the most representative (NI 3 27.5%, NI 5, 28.5%; NI 8 21.97%). Only one sesquiterpene lactone (goyazensolide) was detected, so it constituted 100% of this chemical class.

Hierarchical cluster analysis carried out for the rainfall and temperature variables (Figure 4) showed three readily distinguishable groups, in accordance with the Euclidian distances; the non-hierarchical method K-means confirmed the month distribution suggested by the hierarchical method (Figure 5). According to both methods, the first group was constituted by the hot and rainy months (November, December, January, and February), whereas the second group consisted of cold and dry months (May, June, July, and August). The third group comprised transition months (March, April, September, and October), so the characteristics were intermediate. The Hotelling's T^2 test was performed, so as to compare samples by environmental

Table 3. Values obtained from the analytical methodology validation

Standard compound	MinLD (μg mL^{-1})	MinLQ (μg mL^{-1})	MaxLQ (μg mL^{-1})	Accur. (%)	Intra-CV (%)	Inter-CV (%)
5-*O*-*E*-caffeoylquinic acid	0.5	1.0	500.0	94.06	0.8	1.38
3,4 di-*O*-*E*-caffeoylquinic acid	0.5	1.0	500.0	96.13	0.78	1.17
vicenin-2	0.5	5.0	500.0	93.11	0.73	1.75
Goyazensolide	0.5	2.0	500.0	99.53	0.09	0.91

(MinLD) minimum limits of detection; (MinLQ) minimum limits of quantification; (MaxLQ) maximum limits of quantification; (Accur.) accuracies; (Intra-CV) and (Inter-CV) coefficients of variation for intra-assay and inter-assay precision, respectively.

Table 4. Overall recovery percentages obtained from the analytical method validation after spiking into the matrix 50, 100, and 150 µg of standard compounds

Standard compound	Overall recovery (%)		
	50 µg	100 µg	150 µg
5-*O*-*E*-caffeoylquinic acid	91.3	90.6	90.3
3,4 di-*O*-*E*-caffeoylquinic acid	92.5	91.7	91.3
vicenin-2	90.1	89.7	89.6
goyazensolide	93.4	94.1	93.9

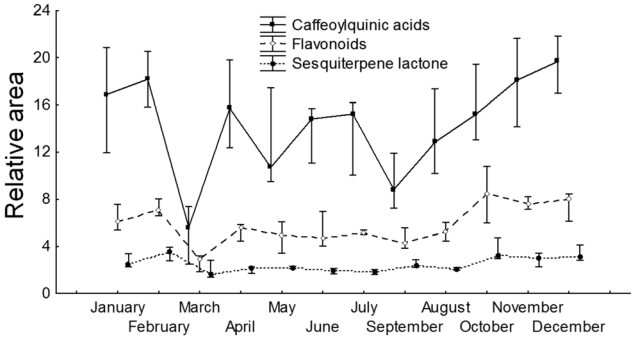


Figure 3. *Mean and standard deviation of the contents of metabolites pertaining to different chemical classes, extracted from Eremanthus mattogrossensis throughout the year. Relative area corresponds to metabolite chromatographic signal area/internal standard area*

Table 5. Mean values and errors of the proportion of each major metabolite representing the respective chemical class, detected in the leaf extract of *Eremanthus mattogrossensis*

Chemical class	Metabolite	Mean percent of total class (%)	Error (%)
Caffeoylquinic acids	3-*O*-*E*-caffeoylquinic acid	4.49	1.07
	5-*O*-*E*-caffeoylquinic acid	14.73	1.86
	3,4-di-*O*-*E*-caffeoylquinic acid	10.63	1.45
	3,5-di-*O*-*E*-caffeoylquinic acid	57.87	2.11
	4,5-di-*O*-*E*-caffeoylquinic acid	12.28	2.00
Flavonoids	Vicenin-2	10.53	1.17
	Flavonoid NI 3	27.55	1.35
	Flavonoid NI 5	28.49	1.85
	Flavonoid NI 8	21.97	1.73
	Flavonoid NI 10	11.47	2.36
Sesquiterpene lactone	goyazensolide	100	0

*NI: non-identified

variable values. This test indicated that there were statistically significant differences among all the values (Figure 5).

Correspondence analysis (CA) was accomplished by grouping samples in accordance with the environmental periods indicated by cluster analyses. In other words, the samples data were organized by

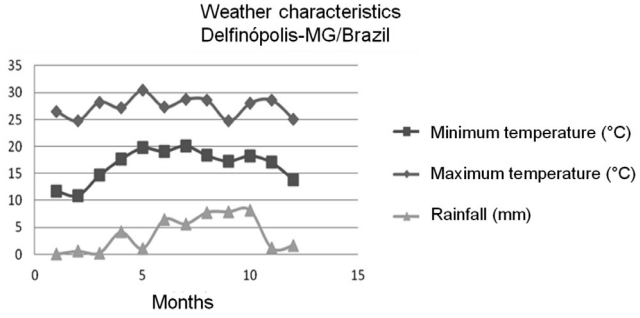


Figure 4. *Weather characteristics: maximum temperature, minimum temperature, and rainfall. On the x-axis, number 1 represents June/2000 and the number 12 represents May/2001*

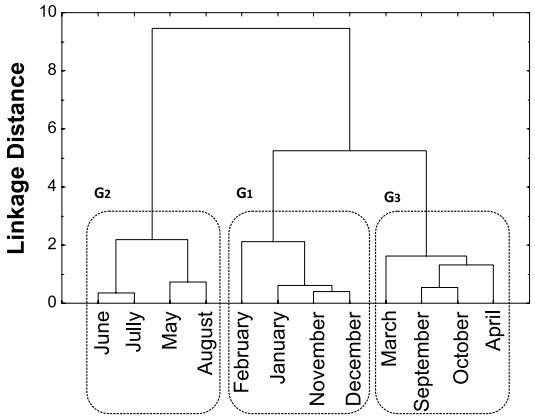


Figure 5. *Hierarchical cluster analysis performed for months groups in accordance with the main weather characteristics, maximum temperature, minimum temperature, and rainfall. G_1, G_2 and G_3 are groups formed by K-means non-hierarchical cluster analysis considering three groups; The Hotelling's T^2 test confirmed statistically significant differences among the groups ($p < 0.001$)*

following the identified seasons (rainy, dry, and transition).

Based on the CA results, first dimension held back 93.48% of data inertia. Because the first dimension (D-1) retained the most important information about samples ordination, it was used alone to understand plant metabolite concentration variation throughout the different seasons. The dry season (G_2) and the rainy season (G1) were plotted in opposite sides of D-1, evidencing strong and inverse correlation between them (Table 6). The plots representing the transition months (G_3) were placed in average positions of D-1, indicating weak correlation with this axis. Hence, the CA results revealed that the core seasons; *i.e.*, the dry and rainy seasons, presented opposite metabolite concentration patterns, whilst the transition months exhibited intermediate conditions.

Interpretation of each metabolite coordinate on D-1 (CA) showed that the caffeoylquinic acids group underwent the widest concentration variation along the season cycle, followed by sesquiterpene lactone (Table 6). Indeed, the plant contained lower amounts of all the chemical compounds in the dry season (G_2), as compared to the other seasons (G_1 and G_3). Although all the metabolites classes existed in higher concentrations during the or close to the rainy season, there was strong correlation between the higher concentrations and the rainy season for the caffeoylquinic acids and flavonoids groups, whereas the sesquiterpene lactone quantity reached its peak at a different timing.

It is known that temperature could control the flavonoids biosynthesis.[18] For *Arnica montana* for example, temperature is the key factor on the alterations of the phenolic compounds in higher altitudes.[19] Another example is the case of *Brassica oleracea*, that high

Table 6. Correlation of season, metabolites class, and concentration categories with the 2-dimensional axis, as calculated by correspondence analysis (CA)

		Concentration categories	D-1 correlation (93.48% of inertia)	D-2 correlation (6.5% of inertia)
Class compounds	Caffeoylquinic acids	a	-0.783	0.059407
		b	-0.128	0.028860
		c	0.046	-0.216025
		d	0.819	0.137997
	Flavonoids	a	-0.664	-0.015160
		b	-0.194	-0.014063
		c	-0.120	0.135802
		d	0.502	0.286948
	Sesquiterpene lactone	a	-0.685	-0.333303
		b	-0.443	0.101027
		c	0.984	-0.161001
		d	0.629	-0.049563
Season	Dry	G_2	-0.615	-0.088640
	Transition	G_3	-0.002	0.237488
	Rainy	G_1	0.678	-0.089438

D-1 and D-2 are the dimension axis calculated by CA; "a", "b", "c", and "d" are increasing concentration categories calculated for metabolites classes. A (Rainy) represents November, December, January, and February; B (Transition) represents March, April, September, and October; C (Dry) represents May, June, July, and September.

levels of UV radiation led to increase the concentration of flavonoids, however the changes induced by UV did not alter the capacity to attract herbivore insects.[20]

In the present work, we could not explain exactly the factors that are responsible for the correlation between the caffeoylquinic acids and flavonoids higher concentrations and the rainy season. However it is known that several factors may alter the secondary metabolism of plants.[1] Some of them are temperature, UV radiation and rainfall. So, we can only suggest that in the rainy season, temperature and UV radiation are more intense and consequently can cause an increase in the concentration of phenolic compounds which have recognized UV protective action.

CONCLUSIONS

A new HPLC-DAD-MS/MS method was developed and validated allowing the analysis of the major metabolites found in the methanol/water extract of the leaves of *Eremanthus mattogrossensis*. Ten specimens were analyzed from the same population on the same day and at the same time and no statistical difference on metabolite production were found. Three of these specimens were analyzed on different days, at one-month intervals, for 21 months and was verified a high correlation between the amount of *E. mattogrossensis* metabolites and seasonality, indicating that environmental properties promote important metabolic responses. Was found a strong correlation between the higher concentrations of caffeoylquinic acids and flavonoids and the rainy season that can be explained by the fact that in the rainy season, temperature and UV radiation are more intense and consequently can cause an increase in the concentration of phenolic compounds.

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