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Environmental factors affecting the concentration of phenolic compounds in *Myrcia tomentosa* leaves

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Abstract: *Myrcia tomentosa* (Aubl.) DC., Myrtaceae, found in Central Brazilian Cerrado and popularly known as "goiaba-brava", belongs to the *Myrcia* genus, which has several species with medicinal properties such as: hypoglycemic, diuretic, hypotensive, antidiarrheal, antimicrobial and antitumor. The present study aimed to analyzed the environmental influence on concentrations of phenolic metabolites in *M. tomentosa* leaves. Compounds assayed in the leaves were: total phenols, tannins by protein precipitation, hydrolysable tannins and total flavonoids and mineral nutrients, while soil fertility was also analyzed, all over during one year. The results were submitted to Pearson Correlation Analysis and *stepwise* Multiple Regression Analysis to investigate the relationship between phenolics and environment data. Analysis of variance and Cluster Analysis allowed indicated a high variability in samples from different sites. The results obtained suggests that content of phenolics from *M. tomentosa* leaves are influenced by environmental factors, particularly some foliar nutrients (N_l, Ca_l and Mn_l), soil nutrients (Ca_s and K_s) and Rainfall.

Introduction

The Myrtaceae family is distributed throughout pantropical and subtropical regions and is constituted by 140 genera and 4000 species. In Brazilian territory, 23 genera with about 1000 species are known (Landrum & Kawasaki, 1997; Souza & Lorenzi, 2005). *Myrcia* comprise around 250 species and is one of the most important genus in the Myrtaceae family, is found in Brazil and many of its species are employed in folk medicine against diabetes, diarrhea, hemorrhages, ulcers of the mouth and several species have diuretic activity (Russo et al., 1990; Mabberley, 1997). Widespread throughout continental South and Central America, species of this genus display important ecological roles in tropical forests (Cardoso & Sajo, 2006; Ramos et al., 2010).

Myrcia tomentosa (Aubl.) DC., Myrtaceae, commonly known as "goiaba-brava", can be found in Brazilian Cerrado, however, only a few papers have described the secondary compounds found in this species (Dianese et al., 1993; Cardoso & Sajo, 2006; Cardoso et al., 2009; Rossatto et al., 2009; Sá et al., 2012). By microdilution bioassay, leaf essential oils of *M. tomentosa* presented moderate activity against Gram positive bacteria, while hexanic and dichloromethanic fractions of leaves showed good activity for *Cryptococcus* sp. with MIC values less than 100 μg/mL (Holetz et al., 2002; Sá, 2010).

Environmental factors, such as soil composition, temperature, rainfall and ultraviolet radiation incidence can affect the concentrations of phenolic compounds (Kouki & Manetas, 2002; Monteiro et al., 2006). Among phenolic compounds, the tannins can be influenced by development of the plant and by environmental changes (Hatano et al., 1986; Salminen et al., 2001). Thus, phenolic compounds and others secondary metabolites represent a chemical interface between plants and environment (Gobbo-Neto & Lopes, 2007). Changes in phenols amounts influence directly the quality of the plant for medicinal application (Santos et al., 2006).

In our early work was evaluated the environmental influence on the concentration of phenolic compounds in stem barks of *M. tomentosa* (Borges et al., 2012). Thus, this work was carried out in order to obtain new information to understanding of the metabolism of phenols in this plant and also provide knowledge to the appropriate cultivation and sampling of the leaves of this plant.

Materials and Methods

Plant material

Leaves of five specimens of *Myrcia tomentosa* (Aubl.) DC., Myrtaceae, were collected in April 2010, August 2010, December 2010 and April 2011 in five

Brazilian cities: Hidrolândia, GO (16° 53' 59.4" S; 49° 13' 29.4" W; 786 m); Nova América, GO (15° 01'11.8" S; 49°52' 32.2" W; 756 m); Crixás, GO (15° 00' 30.2" S; 49° 58' 51.6" W; 755 m); Pires do Rio, GO (17° 12' 35.5" S; 49° 58' 51.6" W; 852 m) and São Gonçalo do Abaeté, MG (18° 20' 27.2" S; 45° 51' 36" W; 919 m). The plants were identified by Dr. José Realino de Paula and vouchers specimens were deposited in the Herbarium of the Universidade Federal de Goiás (UFG), Goiás State, Brazil under code numbers 45518, 43836 and 41318. Samples were air dried (40 °C), pulverized in a knife mill and passed through a 100-mesh sieve respectively.

Samples preparation

Dried and ground leaves (0.75 g) were extracted in a 250 mL erlenmeyer flask with distilled water (150 mL). The mixture was heated to boiling, after kept in water bath at between 80 and 90 °C during 30 min. The contents of the flask were transferred to a 250 mL volumetric flask and the volume was made up with distilled water. The extract was filtered through qualitative filter paper, with first 50 mL discarded. The aqueous extract obtained were used to quantification of total phenols (TP) and for protein precipitation assay (PP).

For the quantification of hydrolysable tannins (HT), dried and ground leaves (0.5 g) were transferred to a 250 mL Erlenmeyer flask with acetone:water 7:3 (3 x 20 mL) and shaken at 60 rpm for 1 h. Next, the residue was decanted and filtered through qualitative filter paper. Each extract was transferred to a rotatory evaporator and the acetone was evaporated (40 °C). The aqueous extract obtained was extracted with ethyl ether (3 x 30 mL) in a separation funnel and the ethereal phase was discarded. Then, the aqueous extract free of grease and chlorophyll was frozen and lyophilized, with the yield calculated.

For the quantification of total flavonoids (Fv), dried and ground leaves (0.25 g) were transferred to a 125 mL flask with methanol:acetic acid 0.02 M 99:1 (50 mL) and the mixture was incubated in water bath under reflux at 90-100 °C during 40 min and then filtered. All samples described early were performed in triplicate.

Colorimetric assays

Total phenolics assay (TP)

Ferric chloride was added to aqueous extract solution under alkaline conditions to result in a coloured complex with phenolic compounds (read at 510 nm), according to Hagerman & Butler (1978) method, adapted by Waterman & Mole (1987a). All solutions were prepared in triplicate. The standards curves were prepared with tannic acid at the dilutions: 0.10, 0.15, 0.20, 0.25, 0.30 mg/mL.

Protein precipitation assay (PP)

The extract solutions were precipitated with Bovine Serum Albumine (BSA) in 0.2 M acetate buffer (pH 4.9) and after centrifugation, the precipitated (containing tannins) was dissolved in sodium dodecyl sulfate/triethanolamine solution, then ferric chloride was added and tannins were complexed (read at 510 nm), according to Hagerman & Butler (1978) method, adapted by Waterman & Mole (1987b). All solutions were prepared in triplicate. The standards curves were prepared with tannic acid at the dilutions: 0.10, 0.20, 0.30, 0.40, 0.50 mg/mL.

Hydrolysable tannins assay (HT)

From the freeze-dried extract was prepared a solution (1.0 mg extract/mL) capable to reacting with potassium iodate over 10 min. After the time had passed, the absorbance was measured at 550 nm (Willis & Allen, 1998). All solutions were prepared in triplicate. The standards curves were prepared with tannic acid at the dilutions: 0.20, 0.30, 0.40, 0.50, 0.60 mg/mL.

Table 1. Climate data for the collection sites over the period from April 2010 to April 2011 - Mean precipitation (mm) and mean temperature (ºC).

Sample	Precipitation (mm)	Temperature (ºC)
H/April/2010	4.58	24.00
NA/April/2010	1.71	26.51
Cr/April/2010	1.71	26.51
PR/April/2010	1.75	23.39
SG/April/2010	3.87	22.75
H/August/2010	-	21.50
NA/August/2010	-	23.67
Cr/August/2010	-	23.67
PR/August/2010	-	21.25
SG/August/2010	-	20.25
H/December/2010	9.87	25.30
NA/December/2010	6.85	27.75
Cr/December/2010	6.85	27.75
PR/December/2010	4.03	24.31
SG/December/2010	12.10	25.33
H/April/2011	1.08	24.10
NA/April/2011	1.28	27.25
Cr/April/2011	1.28	27.25
PR/April/2011	-	24.00
SG/April/2011	1.42	22.75

H: Hidrolândia; NA Nova América; Cr: Crixás; PR: Pires do Rio; SG: São Gonçalo do Abaeté.

Total flavonoids assay (Fv)

The methanolic extract was directly read at 361 nm (Rolim et al., 2005). All solutions were prepared in triplicate. The standards curves were prepared with rutin at the dilutions: 0.010, 0.015, 0.020, 0.025, 0.030 mg/mL.

Climatic data

The average of monthly temperature and daily precipitation were collected from the official site of National Institute for Space Research (Instituto Nacional de Pesquisas Espaciais), (INPE, 2011).

Soil and leaf analysis

Analysis of soil and leaves were performed in Solocria Agricultural Laboratory, following usual techniques (Silva, 2009). Soil samples (500g) were collected at a depth of 20 cm in four locations around each specimen of *M. tomentosa* in all months. The pH was determined in a 1:1 soil/water volume ratio. Calcium (Ca), magnesium (Mg) and aluminum (Al) were extracted with KCl 1M, and phosphorus (P), potassium (K), zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) were extracted with Mehlich's solution. Organic matter (OM), cation exchange capacity (CEC), potential acidity (H+Al), base saturation (V) and aluminum saturation (m) were determined by standard techniques (Silva, 2009).

The quantitative determination of minerals in samples of leaves (15 g) and soil was performed according to the procedure described by Silva (2009). Nitrogen was determined by distillation (semi-micro Kjeldahl method), phosphorus by colorimetry, potassium by flame photometry and sulfur by turbidimetry. Calcium, magnesium, copper, iron, manganese and zinc were determined by atomic absorption.

Statistical analyses

The relationship between phenolic compounds found in leaves of *M. tomentosa* and environmental factors were investigate by *stepwise* Multiple Regression and Pearson's Correlation Analysis implemented using SAS GLM and SAS CORR procedure, respectively (Draper & Smith, 1981). Average multiple comparisons between collections sites and between collection periods were established by a Two-Way Analysis of Variance (ANOVA) complemented by the Tukey's test, and in all comparisons, $p<0.05$ was considered as indicating significance. Cluster Analysis was also applied to study the similarity of samples on the basis of constituent distribution. The hierarchical clustering was performed according to the Ward's variance

Table 2. Levels of mineral nutrients and fertility parameters of soil from each collection site.

Sample	Cu mg/dm³	Fe mg/dm³	Mn mg/dm³	Zn mg/dm³	P mg/dm³	K mg/dm³	Ca mg/dm³	Mg mg/dm³	pH ($CaCl_2$)
H/April/2010	1.60	79.10	62.80	1.30	5.10	69.00	1.40	0.50	5.40
NA/April/2010	1.10	70.40	51.70	1.50	1.80	87.00	1.30	0.50	4.40
Cr /April/2010	0.40	46.50	25.00	1.00	2.10	151.00	3.90	0.70	5.50
PR/April/2010	0.30	100.60	24.40	2.20	2.70	89.00	1.90	0.40	4.20
SG/April/2010	0.40	175.70	12.30	1.30	1.50	27.00	0.90	0.20	4.00
H/August/2010	1.85	76.30	67.60	1.95	4.05	101.50	1.65	0.80	4.90
NA/August/2010	1.20	153.35	52.05	1.75	2.10	91.50	1.85	0.65	4.50
Cr/August/2010	0.60	54.55	27.25	1.10	2.55	175.50	4.10	1.11	5.55
PR/August/2010	2.35	82.20	25.50	1.85	1.95	118.50	1.85	0.80	4.50
SG/August/2010	0.75	128.95	8.00	1.10	1.50	31.00	0.60	0.20	3.85
H/December/2010	2.10	73.50	72.40	2.60	3.00	134.00	1.90	1.11	4.40
NA/December/2010	1.30	236.30	52.40	2.00	2.40	96.00	2.40	0.80	4.60
Cr/December/2010	0.80	62.60	29.50	1.20	3.00	20-	4.30	1.50	5.60
PR/December/2010	1.70	63.80	26.60	1.50	1.20	148.00	1.80	1.20	4.70
SG/December/2010	1.10	82.20	3.70	0.90	1.50	35.00	0.30	0.20	3.70
H/April/2011	1.60	84.70	100.80	2.10	2.10	8-	1.20	0.30	4.60
NA/April/2011	1.50	445.00	31.90	1.40	1.20	52.00	0.70	0.30	4.50
Cr/April/2011	1.70	66.80	11.80	0.50	1.20	14-	1.30	0.80	4.10
PR/April/2011	1.50	56.80	6.20	0.80	0.80	32.00	0.30	0.20	4.80
SG/April/2011	1.30	84.00	11.00	0.60	-	-	-	-	3.90

H: Hidrolândia; NA Nova América; Cr: Crixás; PR: Pires do Rio; SG: São Gonçalo do Abaeté.

minimization method (Ward, 1963). For these procedures were used the softwares SAS (Statystical Analysis System) and Statistica 7.

Results and Discussion

The environmental variables are presented in Tables 1, 2, 3 and 4. The phenolic compounds found in leaves of *M. tomentosa* are shown in Table 5.

From the *stepwise* Multiple Regression, was obtained the following equations with significant (*p*-values less than 0.05) variables (l=leaf and s=soil):

$TP(\%)=15.755 - 0.2033N_l - 0.1994Ca_l - 0.6152Mg_l$ ($r^2=0.7982$; r=0.8934) Equation 1

$PP(\%)=4.8458 - 0.0864Ca_l$ ($r^2=0.4591$; r=0.6775) Equation 2

$HT(\%)=9.8413 + 0.0171Mn_l - 0.4699Rainfall$ ($r^2=0.4622$; r=0.6798) Equation 3

From Table 6, was possible identify that calcium levels found in soil and leaves presented significant negative correlation, weak ($r<0.50$) and strong ($r>0.70$), respectively, with total phenols (TP) and medium correlation ($0.50<r<0.70$) between foliar calcium and tannins by protein precipitation (PP) (Piaw, 2006). This result can be attributed to fact that plants with high Ca levels have a higher resistance to diseases (Yamada, 2004), then it is possible that the plant needs to employ protection mechanisms such increase synthesis of phenols to keep its resistance.

The hydrolysable tannins were negatively correlated with the concentration of K_s (medium correlation). Among the macronutrients, K is the element that shows consistent positive results to reducing the incidence of diseases, suggesting a possible mechanism of compensation for the lack of K, with increasing resistance to pathogens by synthesis of hydrolysable tannins (HT) (Yamada, 2004).

Multiple coefficient of determination (r^2) means the proportion of the total variation that is explained by the overall regression model, so when r^2 is higher, the model fits better to data (Bowerman et al., 2005). The value of r^2 is 0.7982 for equation 1 and shows that there are 79.82% changes in response variables (total phenols), and by comparing models, the equation 1 is the best model that fits the data.

Multiple correlation coefficient (r) is used to look how far the relationship between one dependent variable and a set of independent variables (Ghani & Ahmad, 2010). The value of r is 0.8934 for equation 1 and shows the multiple correlation strength between total phenols and N, Ca and Mg found in leaves. The foliar nutrients was the principal set of independent variables

Table 3. Levels of mineral nutrients and fertility parameters of soil from each collection site.

Sample	H+Al cmolc/dm³	Al cmolc/dm³	CEC cmolc/dm³	O.M. %	M %	V %	Ca/CEC %	Mg/CEC %	K/CEC %
H/April/2010	3.90	-	6.00	1.30	-	34.70	23.40	8.40	3.00
NA/April/2010	3.80	0.70	5.84	2.60	25.74	34.89	22.26	8.56	3.77
Cr /April/2010	2.60	-	7.62	3.70	25.00	65.83	51.18	9.19	5.12
PR/April/2010	4.60	0.70	7.15	2.60	21.67	35.69	26.57	5.59	3.22
SG/April/2010	3.40	0.60	4.58	2.50	33.90	25.83	19.65	4.37	1.53
H/August/2010	4.75	0.25	7.48	2.50	6.51	36.08	22.32	10.35	3.40
NA/August/2010	3.80	0.45	6.56	2.40	15.61	41.32	27.64	9.78	3.60
Cr/August/2010	2.35	-	7.79	3.00	12.50	7-	51.18	13.49	5.60
PR/August/2010	3.55	0.40	6.52	2.15	12.27	46.70	28.60	13.00	4.84
SG/August/2010	3.60	0.60	4.50	2.50	42.16	19.85	13.23	4.46	1.79
H/December/2010	5.60	0.50	8.95	3.70	13.02	37.46	21.23	12.29	3.80
NA/December/2010	3.80	0.20	7.27	2.20	5.48	47.75	33.01	11.00	3.44
Cr/December/2010	2.10	-	8.43	2.40	-	75.06	51.01	17.79	6.05
PR/December/2010	2.50	0.10	5.89	1.70	2.87	57.61	30.56	20.37	6.45
SG/December/2010	3.80	0.60	4.41	2.40	50.42	13.87	6.80	4.54	2.04
H/April/2011	4.40	0.20	8.40	6.50	4.78	47.59	28.57	14.29	4.52
NA/April/2011	3.00	0.40	4.71	1.90	19.05	36.28	25.48	6.37	4.25
Cr/April/2011	5.20	1.40	6.33	1.80	55.34	17.92	11.06	4.74	2.05
PR/April/2011	3.10	0.10	5.58	3.00	3.91	44.40	23.30	14.34	6.45
SG/April/2011	3.50	0.60	4.09	2.40	50.85	14.39	7.33	4.89	1.96

H: Hidrolândia; NA Nova América; Cr: Crixás; PR: Pires do Rio; SG: São Gonçalo do Abaeté.

Table 4. Levels of macronutrients (Nl, Pl, Kl, Cal, Mgl, Sl in g/kg) and micronutrients (Cul, Fel, Mnl, Znl in mg/kg) in the leaves of *Myrcia tomentosa* from each collection site over the period from April 2010 to April 2011.

Sample	N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
H/April/2010	16.00	0.80	12.80	14.00	2.50	1.20	6.00	162.00	420.00	19.00
NA/April/2010	18.00	1.30	9.20	10.50	3.00	1.60	9.00	247.00	308.00	25.00
Cr /April/2010	17.00	1.10	10.40	23.00	4.30	1.40	8.00	162.00	137.00	16.00
PR/April/2010	17.50	1.20	7.60	13.50	4.20	1.50	10.00	183.00	320.00	18.00
SG/April/2010	19.50	1.10	8.00	14.20	1.80	1.50	7.00	141.00	215.00	16.00
H/August/2010	9.60	1.00	5.60	16.10	1.60	1.00	8.00	329.00	340.00	16.00
NA/August/2010	16.80	1.20	0.80	6.30	2.80	1.10	4.00	199.00	87.00	18.00
Cr/August/2010	12.00	1.00	5.00	23.40	3.20	1.00	5.00	257.00	123.00	14.00
PR/August/2010	16.00	1.20	8.00	6.10	4.50	1.40	8.00	217.00	109.00	20.00
SG/August/2010	15.00	0.90	5.20	9.60	2.10	1.10	7.00	165.00	218.00	15.00
H/December/2010	16.60	1.10	6.80	8.00	2.80	1.20	8.00	179.00	172.00	18.00
NA/December/2010	15.80	1.10	6.00	5.80	3.80	1.30	6.00	213.00	168.00	16.00
Cr/December/2010	16.00	1.20	5.20	12.20	4.60	1.20	9.00	99.00	68.00	13.00
PR/December/2010	19.00	1.10	8.80	7.10	2.10	1.10	9.00	131.00	54.00	16.00
SG/December/2010	12.00	1.00	6.00	7.40	2.10	0.80	5.00	116.00	407.00	15.00
H/April/2011	16.80	1.00	8.00	18.00	2.00	1.10	11.00	180.00	500.00	19.00
NA/April/2011	15.60	1.00	8.00	12.00	2.00	1.20	5.00	264.00	485.00	16.00
Cr/April/2011	17.00	1.10	6.60	31.00	2.60	1.50	8.00	144.00	117.00	16.00
PR/April/2011	18.00	1.10	8.40	9.40	1.50	1.00	7.00	174.00	80.00	17.00
SG/April/2011	15.60	1.00	8.00	8.00	1.70	1.10	8.00	147.00	401.00	18.00

H: Hidrolândia; NA Nova América; Cr: Crixás; PR: Pires do Rio; SG: São Gonçalo do Abaeté.

Table 5. Amounts of phenolic compounds in g/100 g dry weight (±standard deviation) of leaves of *Myrcia tomentosa.*

Sample	TP	PP	HT	Fv
H/April/2010	7.68±0.28	4.02±0.18	17.64±0.72	1.46±0.07
NA/April/2010	8.83±0.37	4.19±0.00	12.09±0.17	0.85±0.02
Cr /April/2010	5.86±0.07	2.51±0.06	8.01±0.18	1.28±0.03
PR/April/2010	6.51±0.08	2.74±0.03	15.75±0.12	1.27±0.02
SG/April/2010	8.39±0.18	3.61±0.06	18.02±0.16	1.79±0.04
H/August/2010	9.81±0.22	4.93±0.09	21.56±0.60	1.25±0.02
NA/August/2010	8.80±0.40	4.20±0.00	15.03±0.24	0.85±0.02
Cr/August/2010	6.59±0.15	2.38±0.00	7.93±0.07	1.11±0.02
PR/August/2010	9.14±0.10	4.24±0.20	10.44±0.23	1.83±0.06
SG/August/2010	7.96±0.23	3.33±0.05	12.52±0.20	1.15±0.03
H/December/2010	9.35±0.11	3.83±0.11	8.18±0.15	1.99±0.05
NA/December/2010	9.82±0.39	5.79±0.18	9.19±0.30	3.27±0.11
Cr/December/2010	6.22±0.12	3.10±0.02	6.64±0.12	2.80±0.06
PR/December/2010	7.77±0.35	3.68±0.04	8.88±0.02	2.35±0.05
SG/December/2010	11.05±0.20	4.41±0.10	9.84±0.14	1.63±0.06
H/April/2011	6.96± 0.08	2.98±0.06	15.88±0.17	1.73±0.05
NA/April/2011	8.85± 0.21	3.77±0.18	18.51±0.22	2.31±0.03
Cr/April/2011	4.50±0.07	2.67±0.01	14.82±0.35	2.07±0.00
PR/April/2011	10.89±0.28	4.39±0.11	9.14±0.16	2.72±0.03
SG/April/2011	10.18±0.18	4.05±0.07	10.31±0.20	3.00±0.03

that can influence the levels of total phenols in leaves of *M. tomentosa*. The tannins by protein precipitation and hydrolysable tannins presented medium multiple correlation with its sets of independent variables.

Equations 1 and 2 also showed the negative influence of foliar calcium (Ca_l) over phenolic compounds, due to the negative coefficients obtained in both models, which agree with Pearson's correlation found (Table 6).

Plants with manganese deficiency has the lignification process impaired and phenolic compounds accumulate in the plant tissues (Marschner, 1997; Lin et al., 2005). Moreover, several studies showed the influence of Mn in the schikimic acid pathway, resulting in the biosynthesis of many phenolic compounds, such as flavonoids, tannins and lignin (Santiago et al., 2000; Diaz et al., 2001; Loponen et al., 2001; Lin et al., 2005; Guangqiu et al., 2007). The positive influence of Mn over hydrolysable tannins (HT) found in equation 3 and in Table 6 are in agreement with the previous results about this micronutrient. The potencial base saturation (V) and hydrolysable tannins were negatively corretaled, which reveals that fertile soils are not ideal to increase the synthesis of this metabolite group.

Overall, nutritional stress results in increased production of secondary metabolites, except under sulphur deficiency, when the biosynthesis of secondary metabolites increases, which is consistent with the significant negative correlation found between foliar sulphur and total phenols in Table 6 (Gobbo-Neto & Lopes, 2007; Treutter, 2010).

The Hierarchical Cluster Analysis employing Ward's variance minimizing method showed a highly variability within phenolic compounds of *M. tomentosa* leaves. Figure 1 presents the similarities of the samples on the basis of the distribution of the constituents and this may indicate that the main factor responsible in chemical variability is the collection site with their

Table 6. Values of Pearson's coefficient between environmental variables and phenolic compounds found in leaves of *Myrcia tomentosa.*

	TP	PP	HT	Fv
OM	-0.11517	-0.29846	-0.0765	-0.12997
m	-0.0259	-0.19538	0.040816	-0.10326
V	-0.35979	-0.26345	-0.44365	0.058814
pH	-0.36529	-0.21829	-0.21283	-0.02999
H+Al	0.072197	0.17297	0.4261	-0.18037
Al	-0.11461	-0.09713	0.26918	-0.11438
CEC	-0.40183	-0.22526	-0.21352	-0.07536
Cus	0.079454	0.14465	0.24212	0.060823
Fes	0.19976	0.26069	0.38799	0.22326
Mns	-0.02359	0.17585	0.29728	-0.18366
Zns	0.12052	0.24072	0.18619	-0.16572
P	-0.1885	-0.02027	0.38652	-0.23853
Ks	-0.28945	-0.26528	-0.47866	0.10125
Cas	-0.47639	-0.37524	-0.41946	-0.03442
Mgs	-0.19712	-0.10268	-0.43229	0.18771
N	-0.2347	-0.18922	-0.12277	0.19683
Pf	-0.07167	0.006579	-0.2863	-0.00519
Kf	-0.09021	-0.09153	0.060722	0.10824
Caf	-0.75588	-0.67759	0.198	-0.25834
Mgf	-0.43144	-0.2321	-0.43274	-0.04312
Sf	-0.45361	-0.21472	0.13529	-0.10374
Cuf	-0.34821	-0.30829	-0.03567	0.084079
Fef	0.21308	0.28569	0.42442	-0.33477
Mnf	0.21845	0.10317	0.56255	-0.11515
Znf	0.1902	0.22312	0.17946	-0.29905
Temperature	-0.24626	-0.03541	-0.28829	0.36725
Rainfall	0.24151	0.24987	-0.3463	0.30164

*Significant coefficients (*p*-values less than 0.05).

Table 7. Analysis of phenolic compounds regarding to difference between the collection sites of *Myrcia tomentosa* specimens and collection period.

FT	TPP	TH	Fv	FT	TPP	TH	Fv
$H^{a,b}$	$H^{a,b}$	H^{a}	H^{a}	Apr/2010[a]	Apr/2010[a]	Apr/2010[a]	Apr/2010[a]
NA^{a}	NA^{a}	NA^{a}	NA^{a}	Aug/2010[a]	Aug/2010[a]	Aug/2010[a]	Aug/2010[a]
Cr^{b}	Cr^{b}	Cr^{a}	Cr^{a}	Dec/2010[a]	Dec/2010[a]	Dec/2010[a]	Dec/2010[b]
$PR^{a,b}$	$PR^{a,b}$	PR^{a}	PR^{a}	Apr/2011[a]	Apr/2011[a]	Apr/2011[a]	Apr/2011[b]
SG^{a}	$SG^{a,b}$	SG^{a}	SG^{a}				

Tukey groups (a and b) are given to show significant differences (p<0.05) between specimens and each period. H: Hidrolândia; NA Nova América; Cr: Crixás; PR: Pires do Rio; SG: São Gonçalo do Abaeté.

different environmental factors, the collection time would have less influence in phenols variability between the different months. A two-way ANOVA also showed that the collection site was more important in differences than collection time, as can be seen in Table 7. Total phenols, tannins by protein precipitation and hydrolysable tannins don't showed significant differences. However, April/2010 and August/2010 were different to December/2010 and April/2011, when was considered the total flavonoids (Fv). The differences were greater when considering the means of collecting localities, except for total flavonoids, whose means were not significantly different between the specimens collected. The Analysis of Variance agrees with the results obtained from the Cluster Analysis.

The chemical variability in *M. tomentosa* leaves determined by statistical analyses may reflect environmental influence on phenolic compounds contents, however it may also have been caused by genetic factor in the samples. This work suggests that the main factors capable to influence in phenolic compounds concentration of leaves of *M. tomentosa* are the foliar nutrients such as: Ca_l, Mg_l, Mn_l and N_l. The rainfall also showed capable to change the hydrolysable tannins amounts. The K_s was the only soil nutrient that presented correlation with hydrolysable tannins, exerting a negative influence on this macronutrient. From Cluster Analysis and Analysis of Variance, was possible conclude that the collection site exercised more influence than the collection time, due the similarities of samples of a same site.

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Authors contributions

LLB contributed in the execution of the experimental part of the paper and development of statistical analyses and discussion. SFA gave direct assistance in implementing experimental methods and also in the article writing. BLS contributed with assistance in collecting plant material and had important help in performing assays of tannins by precipitation of proteins. ECC contributed in writing, especially in the discussion of the manuscript. MTFB contributed in writing, especially in the organization of the data. JRP contributed in the identification of specimens, helped in the collection and processing of plant material, beyond the intellectual contribution to the work.

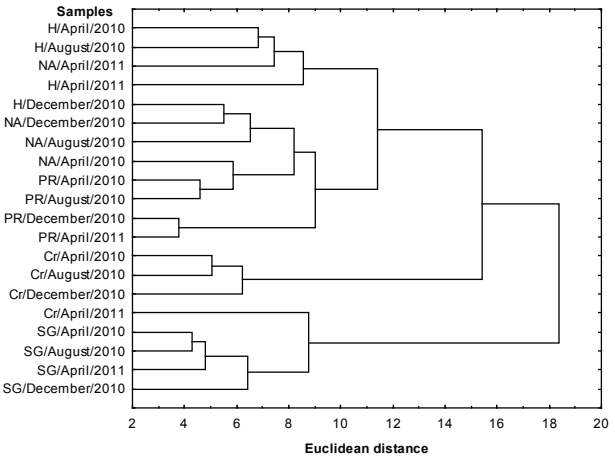

Figure 1. Dendrogram representing chemical composition similarity relationships among leaves of *Myrcia tomentosa*, linking the climatic data, soil nutrients, foliar nutrients and phenolic compounds according to Ward's variance minimization method.

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