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Effect of Environmental Factors on Phenolic Compounds in Leaves of *Syzygium jambos* (L.) Alston (Myrtaceae)

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Abstract

Background: *Syzygium jambos* (L.) Alston, Myrtaceae, is a plant widely used for the treatment of toothache, mouth scores, cough, wound dressing and infectious diseases. Among the metabolite groups identified in leaves of *S. jambos* are the polyphenols, highlighting tannins and flavonoids, related to the pharmacological properties of this plant. Studies on the influence of environmental factors over production of secondary metabolites in *S. jambos* are important because they contribute with knowledge for its cultivation and harvest, besides establish quantitative parameters of secondary metabolites in the plant drug. The aim of this paper was to evaluate the effects of environmental factors on levels of phenolic compounds in *S. jambos* leaves.

Materials and Methods: Total phenols, tannins, flavonoids and mineral nutrients were quantified in leaves, while soil fertility was also analyzed in two different sites and in two months (January and July) from ten specimens (five from each locality).

Results: The data were statistically analyzed and the results have shown that the levels of phenolic compounds in *S. jambos* leaves were influenced by environmental factors, particularly some foliar nutrients (P_l, K_l Ca_l, Na_l, Fe_l, Co_l and Mo_l), soil nutrients (Al_s, K_s, S_s, Na_s and Mn_s) and climatic factors (temperature and rainfall).

Conclusion: The results obtained in this work will be useful for knowledge of the best conditions for leaves collection from *S. jambos*, besides the data analyzed suggested that environmental factors can affect the levels of tannins in this species.

Keywords: Myrtaceae; Secondary metabolism; Environmental factors; Seasonality

Introduction

Syzygium jambos (L.) Alston, also known as *Eugenia jambos* or *Jambosa jambos*, is a medicinal plant traditionally used for the treatment of toothache, mouth scores, cough, wound dressing and infectious diseases [1]. Anti-inflammatory activity was also reported for *S. jambos* leaves extract and its isolated flavonoid glycosides and these properties is closely related to analgesic activity [2-4].

Environmental factors, such as soil composition, rainfall, temperature and humidity, can influence the levels of phenolic compounds in medicinal plants [5-7]. The development of the plant can affect the tannin amounts due a response to the environmental changes [8-10]. Environmental influence over secondary metabolites and studies about chemical variability were investigated in some species from Myrtaceae family [11-15]. In *S. jambos* species, the chemical variability and environmental factors which can influence essential oils were also investigated [16].

Regarding the lack of data on the influence of environmental factors on the production of phenolic metabolites in leaves of *S. jambos*, this study was carried out in order to obtain new data that would inform the appropriate cultivation and sampling of this plant.

Materials and Methods

Plant material

The plant material was collected from ten wild specimens of the plant located in two different municipalities in Goiás state, Brazil: Rio Verde (17°48′33.9″ S; 50°56′ 39,1″ W; 710 m), (17°46′33.6″ S; 50°54′13.2″ W; 688 m), (17°46′27.1″ S; 50°54′52.2″ W; 750 m), (17°46′9.6″ S; 50°54′52.6″ W; 781 m), (17°46′41.2″ S; 50°56′43.1″ W; 758 m) and Nova América (15°01′12″ S; 49°52′33.4″ W; 782 m), (15°01′48.7″ S; 49°51′27.9″ W; 657 m), (15°01′48.6″ S; 49°51′29.9″ W; 652 m), (15°02′58.5″ S; 49°51′53.4″ W; 614 m). The samples were collected in January and July 2011 and received botanic identification by Prof. José Realino de Paula. A voucher specimen has been deposited at the Herbarium of Federal University of Goiás under code number 47579. The samples were air-dried in a chamber at 40°C and ground into a powder.

Colorimetric assays

Total phenolics assay (TP): Ferric chloride was added to each extract under alkaline conditions to result a colored complex with phenols, which was read at 510 nm, following the Hagerman and Butler [17], adapted by Waterman and Mole [18]. All solutions were analyzed in triplicate. The standard curve was constructed with tannic acid at the following dilutions: 0.10, 0.15, 0.20, 0.25 and 0.30 mg/mL.

Protein Precipitation assay (PP): Hagerman and Butler [17] adapted by Waterman and Mole [19] uses Bovine Serum Albumine solution (BSA, 1.0 mg/mL) in 0.2M acetate buffer (pH 4.9). The extract solutions were precipitated with BSA and after centrifugation, the precipitate was dissolved in sodium dodecyl sulfate/triethanolamine solution and the tannins were complexed with ferric chloride and the colored complex was read at 510 nm. All solutions were analyzed in triplicate. The standard curve was

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constructed with tannic acid at the following dilutions: 0.10, 0.20, 0.30, 0.40 and 0.50 mg/mL.

Total flavonoids assay (Fv): The methanolic extract was directly read at 361 nm [20]. 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

Average temperature and average daily precipitation for the periods were collected from the official site of the National Institute for Space Research (Instituto Nacional de Pesquisas Espaciais) [21].

Chemical analysis of leaves and soil

Chemical analysis of soil (500 g) and leaf samples (15 g) was performed at the Solocria Agricultural Laboratory, following standard procedures [22]. The nitrogen (N) was extracted by digestion with H_2SO_4 and catalysts. The minerals Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulfur (S), Copper (Cu), Iron (Fe), Manganese (Mn) and Zinc (Zn) were extracted by digestion with $HClO_4$ and HNO_3.

The soil samples were collected at a depth of 0-20 cm in four locations around each specimen of *S. jambos*, subsequently homogenized and then air dried. The pH was determined in a volume of water-soil at 1:1. Ca, Mg and Al were extracted with KCl 1M, and P, K, Zn, Cu, Fe and 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 methods [22].

The quantitative determination of minerals in leaves and soil was performed according to the methodology described by Silva [22]. 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 *S. jambos* and environmental variables were analyzed by stepwise Multiple Regression and Pearson's Correlation Analysis implemented using SAS GLM (General Linear Models) and SAS CORR (Correlation test) procedure, respectively [23]. 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 minimization method [24]. For these analyses were employed the software's SAS (Statistical Analysis System) and Statistica 7.

Results

Tables 1-4 present the environmental data. The phenolic compounds of leaves are shown in Table 5.

The following equations were obtained by stepwise Multiple Regression with significant variables (p-values less than 0.10 to entry and stay variables in model):

$TP(\%)=15.03+0.0045\ Fe_s+0.0444\ Na_l$ (R^2=0.4540; R=0.6738) (1)

$PP(\%)=4.209+0.2817\ Cu_s+0.0022\ Fe_s+0.3349\ N_l$ (R^2=0.5555; R=0.7453) (2)

$Fv(\%)=-0.3819+0.0503\ Cu_s+0.1324\ Ca_l+0.2889\ Mg_l+0.0989\ Cu_l+0.018\ Zn_l+3.52\ Co_l$ (R^2=0.8736; R=0.9347) (3)

Discussion

The Table 6 showed that potassium levels were negatively correlated with PP and Fv (weak correlation; R<0.5), this result can be attributed to fact that the levels of this macronutrient possess consistent positive capacity to reducing the incidence of diseases, suggesting a mechanism of compensation for the lack of K, capable to increasing resistance to pathogens by synthesis of phenolic compounds [25,26]. This correlation was also observed to *Myrcia tomentosa* species, which belongs to the same family of *S. jambos* [12].

Multiple coefficient of determination (R^2) means the proportion of the total variation that is explained by the regression model, so when R^2 is higher, the model fits better to data [27]. The value of R^2 is 0.8736 for Equation 3, showing that there are 87.36% changes in response variables (total phenols). By comparing models, the Equation 3 is better model fits to the data than the others models.

Multiple correlation coefficient (R) is employed to verify how far the relationship between one dependent variable and a set of independent variables [28]. The value of R is 0.9347 for Equation 1 and shows the multiple correlation strength between flavonoids and Cu_s, Ca_l, Mg_l, Cu_l, Zn_l and Co_l. The foliar nutrients were the principal set of environmental variables that can influence the levels of flavonoids in leaves of *S. jambos*. The total phenols and tannins by protein precipitation presented strong multiple correlation (R>0.7) with its sets of independent variables.

The tannins presented negative correlation with temperature (Table 6), with R=-0.41778. The increased levels of phenolic compounds in the leaves may be related to increased activity of phenylalanine ammonialyase (PAL) at lower temperatures, given the fact that PAL is an important enzyme in the biogenesis of various phenolic compounds, including tannins, which could explain the negative correlation found [29,30]. The same behaviour was observed in bananas that were stored in different temperatures,

Sample	Precipitation (mm)	Temperature (°C)
NA01/Jan/2011	11.93	24.48
NA02/Jan/2011	11.93	24.48
NA03/Jan/2011	11.93	24.48
NA04/Jan/2011	11.93	24.48
NA05/Jan/2011	11.93	24.48
RV01/Jan/2011	8.29	23.9
RV02/Jan/2011	8.29	23.9
RV03/Jan/2011	8.29	23.9
RV04/Jan/2011	8.29	23.9
RV05/Jan/2011	8.29	23.9
NA01/Jul/2011	-	21.62
NA02/Jul/2011	-	21.62
NA03/Jul/2011	-	21.62
NA04/Jul/2011	-	21.62
NA05/Jul/2011	-	21.62
RV01/Jul/2011	-	21.5
RV02/Jul/2011	-	21.5
RV03/Jul/2011	-	21.5
RV04/Jul/2011	-	21.5
RV05/Jul/2011	-	21.5

NA: Nova América; RV: Rio Verde

Table 1: Climate data for the collection sites in the period of January 2011 and July 2011. Mean precipitation (mm) and mean temperature (ºC).

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
NA01/Jan/2011	6.00	310.00	34.00	20.00	1.00	10.00	5.00	1.70
NA02/Jan/2011	5.00	286.00	25.00	23.00	0.90	8.00	5.20	1.60
NA03/Jan/2011	5.00	276.00	24.00	22.00	1.20	10.60	4.90	1.50
NA04/Jan/2011	4.00	240.00	20.00	21.00	1.30	9.80	5.40	1.70
NA05/Jan/2011	7.00	243.00	18.00	19.00	1.20	8.40	4.70	2.20
RV01/Jan/2011	8.00	370.00	25.00	20.00	1.00	8.00	5.20	1.60
RV02/Jan/2011	5.00	305.00	22.00	21.00	1.00	8.00	5.50	1.60
RV03/Jan/2011	6.00	228.00	47.00	16.00	1.10	9.20	6.00	1.40
RV04/Jan/2011	6.00	202.00	61.00	16.00	1.40	9.60	6.20	1.60
RV05/Jan/2011	5.00	294.00	29.00	18.00	1.00	8.80	5.80	1.80
NA01/Jul/2011	4.00	241.00	29.00	11.00	1.00	7.60	5.20	1.70
NA02/Jul/2011	2.00	154.00	20.00	10.00	1.10	6.80	4.20	1.80
NA03/Jul/2011	3.00	367.00	19.00	11.00	1.00	8.00	4.40	1.60
NA04/Jul/2011	3.00	141.00	22.00	14.00	1.10	8.80	6.20	1.50
NA05/Jul/2011	3.00	148.00	24.00	14.00	1.20	8.60	6.50	1.60
RV01/Jul/2011	4.00	270.00	28.00	13.00	1.00	4.80	5.00	2.50
RV02/Jul/2011	4.00	363.00	22.00	14.00	1.10	6.40	6.00	1.80
RV03/Jul/2011	4.00	367.00	45.00	11.00	1.20	8.60	5.20	1.70
RV04/Jul/2011	6.00	345.00	58.00	11.00	1.20	8.00	5.40	1.60
RV05/Jul/2011	2.00	311.00	53.00	13.00	1.40	8.60	5.10	1.80

NA: Nova América; RV: Rio Verde

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

Sample	H+Al cmolc/dm³	Al cmolc/dm³	CEC cmolc/dm³	O.M. %	M %	V %	Ca/CEC %	Mg/CEC %	K/CEC %
NA01/Jan/2011	2.1	0.0	4.54	7.00	0.00	53.66	33.04	8.81	11.23
NA02/Jan/2011	2.3	0.0	6.70	14.00	0.00	65.63	43.28	16.42	5.67
NA03/Jan/2011	1.3	0.0	7.58	33.00	0.00	76.22	47.49	17.15	11.35
NA04/Jan/2011	1.8	0.0	11.07	67.00	0.00	88.27	73.17	9.03	5.87
NA05/Jan/2011	2.7	0.0	5.87	8.00	0.00	54.03	32.37	17.04	4.26
RV01/Jan/2011	4.0	0.1	6.82	8.00	3.44	41.39	26.39	13.20	1.61
RV02/Jan/2011	2.9	0.0	8.35	12.00	0.00	65.24	47.90	15.57	1.56
RV03/Jan/2011	1.9	0.0	8.26	11.00	0.00	76.95	61.74	10.90	4.00
RV04/Jan/2011	2.6	0.0	6.49	10.00	0.00	59.96	40.06	16.95	2.62
RV05/Jan/2011	2.8	0.0	9.30	14.00	0.00	69.85	50.54	13.98	5.05
NA01/Jul/2011	2.6	0.1	6.52	23.00	2.50	60.15	33.74	10.74	15.34
NA02/Jul/2011	2.5	0.4	4.46	14.00	17.17	43.86	29.15	6.73	7.40
NA03/Jul/2011	2.0	0.0	7.46	18.00	0.00	73.21	45.58	16.09	11.26
NA04/Jul/2011	1.7	0.0	9.62	25.00	0.00	82.30	49.90	19.75	12.47
NA05/Jul/2011	2.0	0.0	10.33	28.00	0.00	80.60	46.47	19.36	14.52
RV01/Jul/2011	2.7	0.0	6.34	12.00	0.00	57.39	42.59	12.62	2.05
RV02/Jul/2011	2.7	0.0	7.11	14.00	0.00	62.07	47.82	12.66	1.41
RV03/Jul/2011	1.7	0.0	7.72	12.00	0.00	78.00	60.88	10.36	6.48
RV04/Jul/2011	2.2	0.0	5.55	13.00	0.00	60.41	50.45	7.21	2.52
RV05/Jul/2011	2.2	0.0	5.45	18.00	0.00	59.61	45.87	7.34	6.24

NA: Nova América; RV: Rio Verde

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

and PAL activity was increased in lower temperatures [31]. In other study with tomato plants (*Lycopersicon esculentum* L.), the thermal stress caused highest phenylalanine ammonia-lyase activity, and this results are in agreement with the trend found in our paper [32]. To other hand, this sensibility to climatic changes was also observed in phenolic compounds present in grape skins, in which warm temperatures presented positive correlation with the levels of phenolic compounds [33]. Also, related to PAL, the micronutrient Cu is linked to production of phenolic compounds in plants, because it is capable to activate the PAL pathway, and this may explain the positive correlation between Cu present in soil with tannins and flavonoids observed in Table 6. Another explanation suggested of the increased levels of phenolic compounds in these tissues, is that this is associated with a mechanism of tolerance to Cu, since Cu is a catalyst for redox reactions that can generate free radicals harmful to the plant; consequently, increased levels of phenolic compounds may have two goals: to decrease the concentration of free Cu in plant tissue by the reaction of this with phenols, and to minimize the deleterious effects of free radicals formed, through the antioxidant reactions of the phenolic compounds [34].

Sample	N	P	K	Ca	Mg	S	Cu	Fe	Mn	Zn
NA01/Jan/2011	12.00	1.00	10.00	5.00	1.70	1.00	6.00	310.00	34.00	20.00
NA02/Jan/2011	12.20	0.90	8.00	5.20	1.60	1.30	5.00	286.00	25.00	23.00
NA03/Jan/2011	12.00	1.20	10.60	4.90	1.50	1.00	5.00	276.00	24.00	22.00
NA04/Jan/2011	12.80	1.30	9.80	5.40	1.70	1.20	4.00	240.00	20.00	21.00
NA05/Jan/2011	12.00	1.20	8.40	4.70	2.20	1.10	7.00	243.00	18.00	19.00
RV01/Jan/2011	13.60	1.00	8.00	5.20	1.60	1.00	8.00	370.00	25.00	20.00
RV02/Jan/2011	12.50	1.00	8.00	5.50	1.60	1.10	5.00	305.00	22.00	21.00
RV03/Jan/2011	13.20	1.10	9.20	6.00	1.40	1.10	6.00	228.00	47.00	16.00
RV04/Jan/2011	14.00	1.40	9.60	6.20	1.60	1.20	6.00	202.00	61.00	16.00
RV05/Jan/2011	12.60	1.00	8.80	5.80	1.80	1.10	5.00	294.00	29.00	18.00
NA01/Jul/2011	14.00	1.00	7.60	5.20	1.70	1.20	4.00	241.00	29.00	11.00
NA02/Jul/2011	13.00	1.10	6.80	4.20	1.80	1.40	2.00	154.00	20.00	10.00
NA03/Jul/2011	12.60	1.00	8.00	4.40	1.60	1.60	3.00	367.00	19.00	11.00
NA04/Jul/2011	13.40	1.10	8.80	6.20	1.50	1.40	3.00	141.00	22.00	14.00
NA05/Jul/2011	12.80	1.20	8.60	6.50	1.60	1.50	3.00	148.00	24.00	14.00
RV01/Jul/2011	14.20	1.00	4.80	5.00	2.50	1.60	4.00	270.00	28.00	13.00
RV02/Jul/2011	14.40	1.10	6.40	6.00	1.80	1.80	4.00	363.00	22.00	14.00
RV03/Jul/2011	13.00	1.20	8.60	5.20	1.70	1.90	4.00	367.00	45.00	11.00
RV04/Jul/2011	13.20	1.20	8.00	5.40	1.60	1.60	6.00	345.00	58.00	11.00
RV05/Jul/2011	15.00	1.40	8.60	5.10	1.80	1.80	2.00	311.00	53.00	13.00

NA: Nova América; RV: Rio Verde

Table 4: Levels of macronutrients (N_l, P_l, K_l, Ca_l, Mg_l, S_l in g/kg) and micronutrients (Cu_l, Fe_l, Mn_l, Zn_l in mg/kg) in the leaves of *Syzygium jambos* from each collection site in January 2011 to April 2011.

Sample	TP	PP	Fv
NA01/Jan/2011	21.151 ± 0.003	9.985 ± 0.010	2.151 ± 0.012
NA02/Jan/2011	21.134 ± 0.002	10.133 ± 0.011	1.914 ± 0.006
NA03/Jan/2011	19.766 ± 0.004	9.068 ± 0.008	1.910 ± 0.029
NA04/Jan/2011	20.750 ± 0.001	8.421 ± 0.008	2.006 ± 0.013
NA05/Jan/2011	23.784 ± 0.003	9.675 ± 0.015	2.218 ± 0.018
RV01/Jan/2011	20.143 ± 0.003	9.897 ± 0.005	2.364 ± 0.014
RV02/Jan/2011	18.614 ± 0.002	9.818 ± 0.013	2.153 ± 0.003
RV03/Jan/2011	20.582 ± 0.002	9.524 ± 0.005	2.272 ± 0.005
RV04/Jan/2011	18.147 ± 0.002	9.675 ± 0.008	2.165 ± 0.008
RV05/Jan/2011	19.453 ± 0.005	9.259 ± 0.010	2.216 ± 0.004
NA01/Jul/2011	21.748 ± 0.003	11.647 ± 0.008	2.265 ± 0.043
NA02/Jul/2011	23.047 ± 0.005	9.535 ± 0.006	1.815 ± 0.005
NA03/Jul/2011	20.397 ± 0.003	10.269 ± 0.005	2.061 ± 0.007
NA04/Jul/2011	22.912 ± 0.003	10.147 ± 0.010	2.156 ± 0.008
NA05/Jul/2011	21.629 ± 0.003	11.111 ± 0.022	2.448 ± 0.006
RV01/Jul/2011	18.860 ± 0.002	10.239 ± 0.012	2.372 ± 0.726
RV02/Jul/2011	19.949 ± 0.003	10.721 ± 0.024	2.480 ± 0.005
RV03/Jul/2011	21.591 ± 0.004	10.109 ± 0.011	2.169 ± 0.003
RV04/Jul/2011	20.658 ± 0.002	8.341 ± 0.013	2.200 ± 0.040
RV05/Jul/2011	20.329 ± 0.005	9.780 ± 0.013	2.134 ± 0.012

NA: Nova América; RV: Rio Verde; TP: total phenols content; PP: tannins by protein precipitation assay; Fv: total flavonoids

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

The nutrient Fe_s showed positive correlation with TP and PP, and according Jin et al. [35] phenolic compounds may complex with Fe^{3+} and be transported to other tissues, facilitating its mobilization between different tissues, and also participating in reduction reactions of Fe^{3+} to Fe^{2+}, aiding reductase-type enzymes.

The Hierarchical Cluster Analysis employing Ward's variance minimizing method showed a highly variability within phenolic compounds of *S. jambos* leaves. From Figure 1 it can be seen 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 time, due the similarities of samples of a same site and the collection site would have less influence in phenols variability between the two different localities.

Conclusion

This paper suggests that there is a relevant influence of environmental factors over production of phenolic compounds in the leaves of *S. jambos*, with foliar nutrients (P_l, K_l Ca_l, Na_l, Fe_l, Co_l and Mo_l) soil nutrients (Al_s, K_s, S_s, Na_s and Mn_s) and climatic factors (temperature and rainfall), being the main variables that may change the levels of phenolic compounds in this plant tissue. From the data obtained, the best conditions of collection can be established for leaves of *S. jambos*.

	TP	PP	Fv
Cas	-0.10705	-0.27518	0.005473
Ms	-0.02181	0.23104	0.19327
Als	0.37711	0.036006	-0.3875**
HAl	-0.23139***	0.17572	0.40273**
Ks	0.40544**	0.37155***	-0.03278
Ss	-0.38337**	0.14683	0.37144***
Nas	0.33869***	0.042196	-0.21794
Zns	-0.09854	-0.26323	0.10575
Bs	0.020883	-0.25372	0.00098
Cus	-0.19116	0.49054*	0.36616***
Fes	0.58551*	0.42248**	-0.34742***
Mns	0.50044*	0.39947**	-0.17427
Nl	-0.27365	0.2824	0.43661**
Pl	-0.03023	-0.35143***	-0.06747
Kl	0.02338	-0.38467**	-0.34668***
Cal	-0.25612	0.15188	0.55497*
Mgl	0.036881	0.10184	0.23637
Sl	0.057513	0.2213	0.19748
Nal	0.17358	0.18636	0.40286**
Cul	-0.19142	-0.2768	0.23941
Fel	-0.38217**	-0.10146	0.16464
Mnl	-0.32657	-0.25279	0.099418
Znl	-0.22435	-0.30987	-0.1938
Col	0.2682	0.40508**	0.22787
Mol	0.24594	0.43497**	0.24723
Temperature	-0.17664	-0.41778**	-0.27652
Rainfall	-0.1406	-0.42803**	-0.30053

*Significant at: *1%, **5% and ***15%. TP: total phenols content; PP: tannins by protein precipitation assay; Fv: total flavonoids

Table 6: Values of Pearson's coefficient between environmental variables and phenolic compounds found in leaves of *Syzygum jambos.*

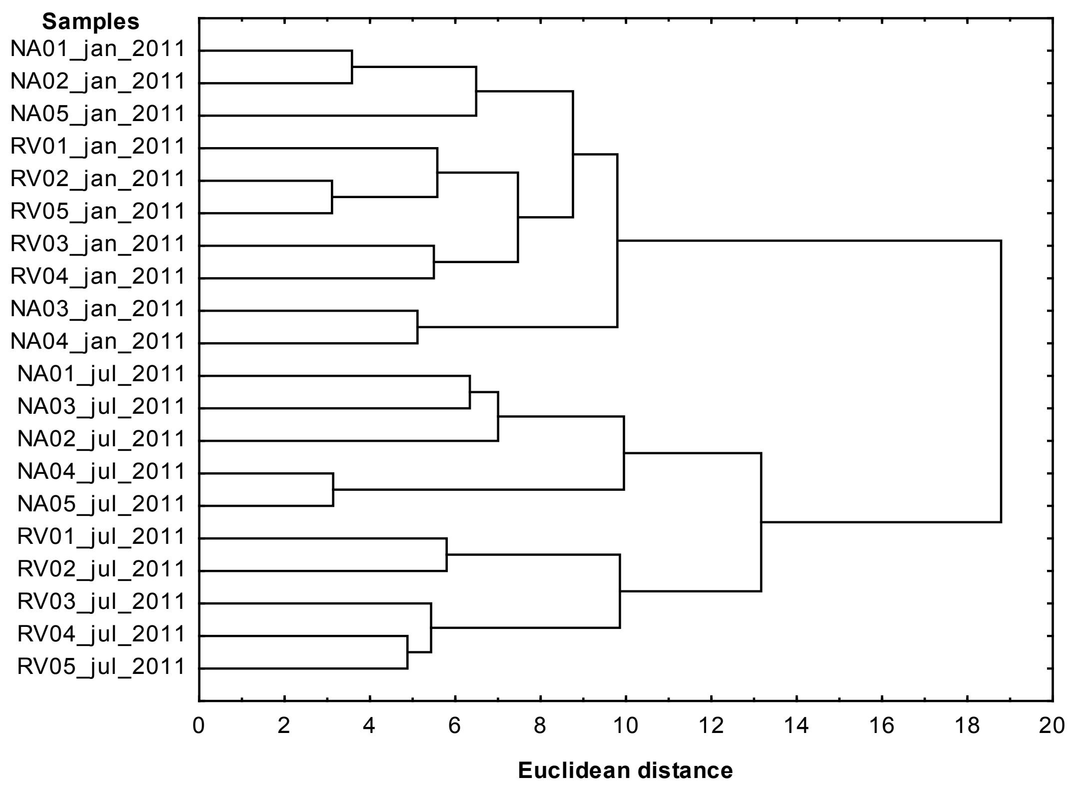

Figure 1: Dendrogram representing chemical composition similarity relationships among leaves of *S. jambos*, linking the climatic data, soil nutrients, foliar nutrients and phenolic compounds according to Ward's variance minimization method.

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