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RESEARCH ARTICLE

CHEMICAL AND THERMAL EVALUATION OF GRASS MATE (ILEX PARAGUARIENSIS ST. HIL.) NATIVE PROGENIES, FROM KAIOWÁ AND GUARANI INDIGENOUS COMMUNITIES/MS

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ABSTRACT

This study aimed thermal evaluation (TG/DTG and DSC), mineral elements quantification by atomic absorption and caffeine content of native progenies of grass mate (*Ilex paraguariensis* St.), in order to complement the genetic evaluation of these progenies at field to allow the selection of more productive ones for sustainable exploitation of natural resources by the indigenous community. The thermogravimetric analysis (TG/DTG) corresponds to the mass thermal decomposition process, which began at 150°C and had a sharp peak in exothermic reaction of 450°C at the end of the process. The curves (DSC) have similar thermal behavior in the process of thermal decomposition that varies in temperature range of 150°C to 450°C. All progenies have an initial mass loss of around 10%, due to the water removal and other volatile compounds. The analysis of minerals performed by the atomic absorption spectrophotometry did not show significant differences between the examined elements (Na, Mn, Zn, Cu, Ca, Fe, Mg, and K), however the progeny number 7 presented significative mean values of potassium (± 2681.23), and it is observed, also, similarity between the blocks. The concentration of caffeine content varied in the range of 6.566 mg/g to 13.630 mg/g, with emphasis on blocks 1 and 4, there was no significant deviation among treatments and calculated R value was 0.9945 between 0.002 to 0.007 mg/g, which increases the energetic potential of plant material. So, grass mate represents an alternative of employment and income to the indigenous community.

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INTRODUCTION

Grass-mate (*Ilex paraguariensis* St. Hil.), is an important native product at the cultural, social and economic context from the state of Mato Grosso do Sul – Brazil, and also integrate the quotidian of the Kaiowá e Guarani community. Belonging to the family Aquifoliaceae, the genera *Ilex* it has more than de 550 species and it is recognized for its properties as anti-inflammatory, therapeutics and diuretics. Originary from the South America, its natural area of occurrence is restrict to three countries: Argentina, Brazil and Paraguay, all over an superficial area of about 540.000 Km², between the latitudes 21º to 30º Sul and longitudes from 48º 30' to 56º 10' West, and altitudes varying between 500 a 1000 meters (Scheidt, 2006). Such specie can occur at isolated points, out of these limits, in subtropical and temperate regions of South America (Da Croce, 2002). In Brazil, this area is dispersed mainly at the states of Rio Grande do Sul, Santa Catarina, Paraná, Mato Grosso do Sul, São Paulo e Minas Gerais, extended all over 450000 Km². According to Carneiro *et al*. (2006), grass-mate represents an expressive participation at socioeconomic national aspects, mainly at the south and centre-west of the country, where it represents one of the most important economic cycles, turning into a fact of great value to hold the man at the country area, as far as it is commercialized by small producers. The interest by natural products, the isolation and characterization of their constituents using analytical methods add economic value to the product and make available specific application and therapy. According to Esmelindro *et al*. (2002) many studies are permitting expand the options due to the physical-chemical composition of the

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grass-mate, adding biotechnological studies with protein and functional molecules, as far as the extraction of compounds with industrial interest. According to Bastos *et al*. (2006) the chemical-physical investigation related to grass-mate were initiated by Trommsdorff in 1836, constantan the presence of many resinous substances, dying material, tannic acid, among other elements. The identification of the main alkaloid, the caffeine, occurs in 1843 by Stenhouse. Analysis and studies about grass-mate has reveled a composition that identify many properties that bring benefits to human being, because the material has alkaloids (caffeine, methylxantine, teofiline e teobromine), tannins (folic and caffeic acids), vitamins (A, B1, B2, C e E), mineral salts (aluminum, calcium, phosphor, iron, magnesium, manganese and potassium), proteins (essential amino acids), sugars (fructose, glucose, rafinose and sucrose), grasses (essentials oils and wax substances), besides it has cellulose, dextrin, saccharine and gums.

Other important aspect related to the chemical and thermal characterization of the leaves, objecting the use of these bioactive products at commercial and therapeutic application, because the use of medicinal plants at Brazil helps significantly the basic health care and for the therapy of common infections, where many plants are used without any evidence of their efficacy. (Asolini *et al*., 2006; Gnoato *et al*., 2007). Facing this exposition, the present work have the objective of evaluate the thermal stability and heat flux involved at the thermal decomposition process, evaluate the analysis of mineral using atomic absorption and the quantitative determination of caffeine at native progenies of grass-mate (I*lex paraguariensis* St. Hil.), at indigenous reserve of Kaiowá and Guarani.

MATERIALS AND METHODS

The progenies of native grass-mate (*Ilex paraguariensis* St. Hil) were finding installed in blocks sparsely by chance at experimental area of indigenous reserve of Kaiowá and Guarani, at the district of Caarapó at the State of Mato Grosso do Sul. The total area of the reserve has 3600 hectares, localized at the latitude 22° 35' South and longitude 55° 00' West (Fig.1). The experimental design to collect the grass-mate samples were the blocks randomized, with 7 treatments, 5 repetitions and 10 plants by parcel. The parcels sizes were of 4 m^2 (2 X 2).

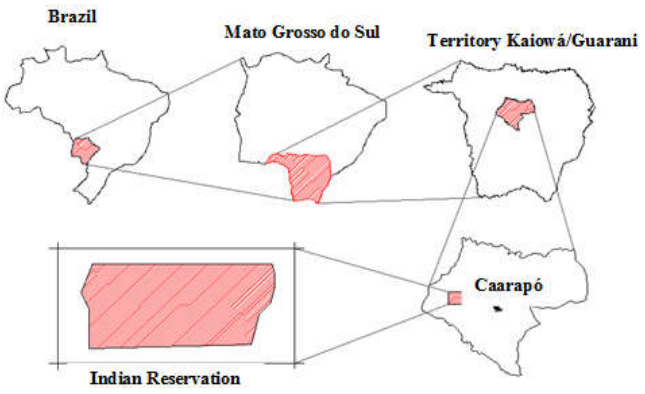

Fig. 1. ***Localization of the Indigenous Reserve of Caarapó/MS***

Grass-mate Leaves Chose, Harvest and Drying

The vegetal part selected for the study of the thermal behavior, atomic absorption of the mineral and quantitative determination of caffeine at the grass-mate (*Ilex paraguariensis* St. Hil.), were the leaves of the native progenies selected for genetic improvement program. The samples were collected by chance, at various haughty of the tree, cleaned and dried in drier with forced air circulation at 40°C for 72 hours. After drying, the samples were milled until 35 meshes. About 10 milligrams of the samples were used for the analysis of the thermal behavior; 3 grams were used for the determinations of minerals (K, Ca, Na, Mg, Fe, Mn, Cu e Zn) and 2 grams for the quantitative determination of caffeine.

Thermal behavior

***Thermogravimetry- Derivate Thermogravimetry (TG-DTG)*:** The curves TG-DTG were obtained at a num "Shimadzu Thermal Analysis System TA- 50H", composed by a microbalance with the maximal capacity of 2000 mg and sensitive of 1 ug; oven TGA-50H, able to operate from environmental temperature to 1400°C, controlled by a micro processing. The thermo-gravimetric curves were done in nitrogen atmosphere (N2), using cadinhos de alumina (Al_2O_3).

Differential Sploratory Calorimetry (DSC): The curves DSC were obtained at the equipment DSC-50 - Shimadzu, able to operate from environmental temperature to 750°C, that takes part of the "Shimadzu Thermal Analysis System TA- 50H", using as sample support, cadinho de alumina without cap, and as reference, a similar cadinho, empty, in nitrogen atmosphere.

***Mineral Analysis by Atomic Absorption*:**The analyses of metals (Ca, Mg, Cu, Mn, Zn e Fe) were done at an Atomic Absorption Spectrum (FAAS) at the absorption mode and for the determination of K and Na at the emission mode. The quantitative determination of metals at the samples was based at the calibration curve obtained with the standard solution (external standard method). To avoid possible interferences at the analysis of Ca and Mg, it was added lanthanum chloride 1% to acid solutions (samples and standard solutions). 3 g of the samples were submitted to carbonization at electric plate at 350°C. After, it was incinerated at a muffle at 700°C for a period of more or less 7 hours. The ashes produced were dissolved into 2 mL of nitric acid concentrated and 2 mL of H_2O_2 30% in volume. This solution was heated till the clarification of the sample. The final solution was filtered on quantitative filter, and the volume was lead to 50 mL with deionized water. All determinations were done in triplicate for the samples and for the standard solutions. The results of macro and micro nutrients were submitted to the variance analysis and the average of the treatments compared statistically by the Duncan test, using a computational program (software SANEST).

Quantitative Determination of Caffeine

The determination of the amount of caffeine can be divided into three steps

***Caffeine Extraction*:** It was used 2 g of material for each sample, which was heat in water bath at 80°C during 15 minutes with 4 mL of sulfuric acid, after it was added 50 mL of hot water and it was kept for more 15 minutes at 80°C. Then the sample was filtered at hot temperature, refreshed and neutralized with NaOH solution at 40%. At the end of this step, it was done the extraction with chloroform (4 turns with 20 mL each). The obtained extract was desiccated with anidro sodium sulfate, collected in volumetric flask of 100 mL and the volume was completed with chloroform.

Reference solution Preparation: 10 mg de caffeine (Merck, p.a) was dissolved into 100 mL de chloroform. Solutions contemning 2.5; 5.0; 7.5; 10.0; 15.0 and 20.0 mg of caffeine per 50 mL in chloroform were prepared. It was measured the absorbance of these solutions at 290.5 nm (wave length), using chloroform as blanch.

***Quantitative Determination of caffeine*:** 1 mL of the extract was taken and diluted into25 mL of chloroform, in duplicate. Then it was measured the absorption of the solution in spectrophotometer (AQUAMATE), using cubes of quartz de 1 cm de thickness, according to the methodology specified by Institute Adolfo Lutz (2005), adapted by Valduga, 1994, and Esmelindro *et al*., 2002. In order to evaluate the linearity of the response and the detention limit of these spectrometric conditions it was constructed the calibration curve for the analysis of caffeine in chloroform. The curve (fig. 2) correspondent to the absorption of caffeine solutions allow verify that it was not find a significant deviation of the linearity, being the correlation factor r^2 = 0,9945 between 0,002 a 0,007 mg/mL of caffeine.

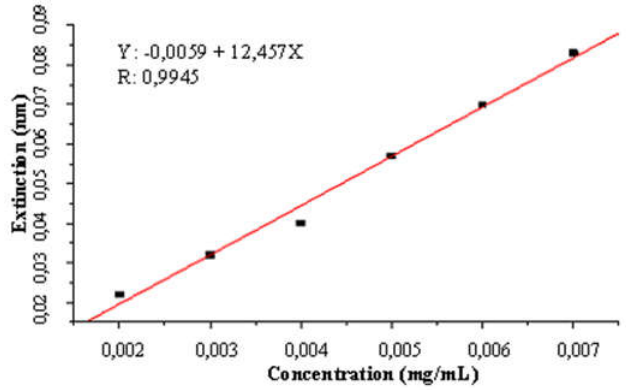

Fig. 2. Calibration curve for the analysis of caffeine in chloroform

RESULTS AND DISCUSSION

It were analyzed leaves obtained of selected progenies at the phase of mudas production of native grass-mate, to obtain data about the thermal stability and the flux of heat involved at the thermal decomposition process of this vegetal biomass, thought the "Thermogravimetry – Derived Thermogravimetry" (TG-DTG) and Differential Sploratory Calorimetry (DSC). The samples were collected by chance, dried, milled to the analysis of the thermal behavior. Fig. 3 shows the curves TG - DTG of the sample of grass-mate *in natura*. The curve TG show the loss in mass in three steps: the first curve correspond to the dehydrates process (10%) from environmental temperature to 150ºC. From this temperature to 450ºC there were two loses subdivided in two more steps, but in consecutive reactions according to the curve DTG, until the obtention of the ash content, as can be observed for the progenies 4, 5 e 2. The curve DSC for the grass-mate *in natura* shows broad endotherms correlated to the dehydratation, showed at the curves TG e DTG, followed by two exotherms also in accordance to the mass loss observed.

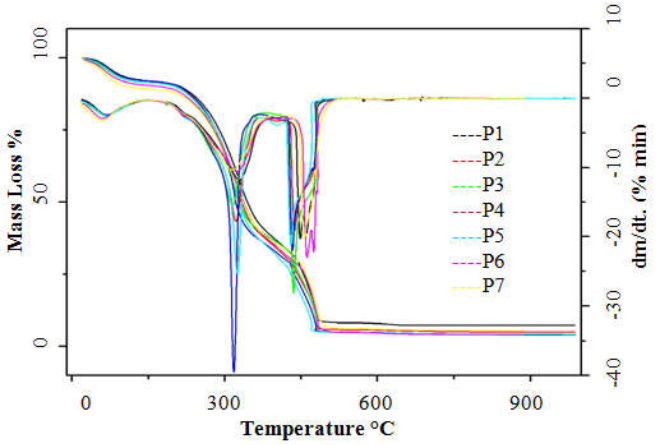

Fig. 3. Curves of TG e DTG related to the samples of grass-mate in natura, obtained at temperature between 50 °C to 900 ºC in dynamic atmosphere of synthetic air with the flow of 100 mL⁄min, heating rate of 20 °C. min^{-1}, support of α-Al_2O_3.

Figure 4 shows the curves DSC with similarity at the thermal behavior; otherwise the progenies 6, 7 e 3 present final mass losses around 450°C at the decomposition process. All progenies present an initial mass lost around 10%, due to water elimination and other volatile compounds of the grass-mate in low quantities, probably organic compounds with low molecular weight. At the sequence two consecutive mass loses beginning in range temperatures of 280 °C to 320 °C that are attributed to the samples thermo decomposition process. At 450ºC there is a strong exothermic reaction provoked by the over heat plus the heat generated by the sample in oxidant atmosphere, causing a bowling at the TG curve. On the other hand the curves DSC of these samples, at this same range of temperature show a big exothermic pick, provoked by the same reason.

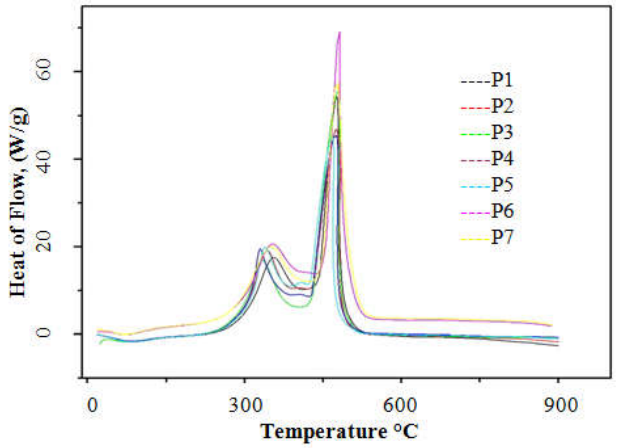

Fig. 4. DSC Curves related to the samples of grass-mate in natura, obtained at temperature between 50 °C to 900 ºC in dynamic atmosphere of synthetic air with the flow of 100 mL⁄mi , heating rate of 20 °C. min^{-1}, support of α-Al_2O_3

According to Oliva (2007), the concentration of nutrients at the grass-mate plant, varies significantly function of the material origin and soil nutrient concentration, weather, specie, plant age, harvest season and mainly the genetic characteristics. The results obtained related to the mineral contents determination done by atomic absorption spectrophotometry among the progenies of grass-mate were considered satisfactory to the elements Na, Mn, Zn, Cu, Ca, Fe, Mg e K. But, there are significant differences among the progenies. These differences can be attributed to the allow ability of the reminiscent native genetic material. According to Heinrichs and Malavolta (2001) the analysis done on grass-mate showed that this plant has many vitamins, along with calcium, magnesium, sodium, iron and fluor, among other elements essential to life. Summarizing, the main difference among the chemical-physics parameters measured by atomic absorption spectrophotometry (Table 1) was statistically significant among the native progenies analyzed, mainly the average values of Mn, Zn, Ca e Fe. On the other hand this evaluation was spotted in analyze the present data, because different methods were used to dissolve the solids obtained at the total digestion of the samples, and also the age of the plants represents an important effect of differentiation of the leaves concentration.

noticed that all progenies have average contents of Ca satisfactory, but the progenie P1 showed the highest concentration with 982.90 mg kg^{-1} and the progenie P3 presented the lowest concentration with 590.40 mg kg^{-1}. According to Malavolta *et al*. (1997), this nutrient is essential to keep the structural integrity of the cellular membrane and walls. Souza *et al*. (2008) describes that this behavior can be occurred due to the function of the characteristic of the nutrient (Ca), presenting low mobility at the plant due to its immobilization at the cellular wall and at the phloem, mainly at the form of calcium oxalate. Fe presents a different behavior comparing to the other elements. As it is observed, the average contents are high, around 11.99 mg kg^{-1} and there are a few variations among the progenies and among the blocks analyzed. In the case of Fe, we should consider that the specific absorptions mechanisms of this element are closely linked with the genotype of the plant (Jolley and Brown, 1994). When it comes to the ashes, the results show no significative differences among the minimum and maximum contents, on the other hand it is observed a small reduction of the minerals obtained at this analysis, is possibly related to the methodology used; many papers report contents measured from classical gravimetric analysis thought calcinations of the

Table 1. Average amount of the minerals (mg kg^{-1}) done by atomic absorption spectrophotometry, of the progenies of the native grass-mate cultivated at the indigenous reserve Kaiowá and Guarani, Caarapó-MS

Progenies	Elements								
	Ashes %	Na	Mn	Zn	Cu	Ca	Fe	Mg	K
P1	5.07^{b}	86.00^{e}	286.57^{e}	2.03^{b}	0.46^{bc}	982.90^{f}	10.70^{b}	734.33^{e}	478.23^{a}
P2	5.87^{c}	40.63^{ab}	234.20^{d}	2.20^{c}	0.34^{a}	717.30^{c}	10.63^{b}	482.20^{a}	802.63^{d}
P3	6.10^{c}	38.50^{a}	125.33^{a}	2.93^{e}	0.31^{a}	590.40^{a}	13.30^{c}	525.93^{b}	1198.00^{c}
P4	4.97^{ab}	42.87^{b}	175.30^{b}	1.67^{a}	0.46^{bc}	723.73^{c}	12.90^{c}	484.80^{a}	955.73^{e}
P5	4.33^{a}	39.30^{a}	184.43^{c}	2.77^{d}	0.30^{a}	703.37^{b}	9.47^{a}	499.86^{ab}	640.50^{b}
P6	5.20^{b}	54.70^{d}	176.23^{b}	3.20^{f}	0.42^{b}	858.30^{e}	15.80^{d}	542.30^{c}	1282.23^{f}
P7	5.43^{b}	46.90^{c}	185.87^{c}	2.83^{de}	0.49^{d}	791.97^{d}	11.17^{bc}	645.46^{d}	626.00^{b}
Average	5.28	49.84	195.41	2.51	0.39	766.85	11.99	559.26	686.76
CV%	11.18	33.91	26.19	22.07	19.86	16.42	17.87	17.03	35.53

Average with the same letters, are not statistically different at the level of 5% at the test of Duncan, considering the native progenies of grass-mate.

Table 2. Average content of caffeine (mg/g) measured by spectrophotometry at UV, of the progenies of native grass-mate cultivated at the indigenous reserve of Kaiowá and Guarani, District of Caarapó-MS

Progenies	Treatments				
	Block 1	Block 2	Block 3	Block 4	Block 5
P1	10.58^{ab}	6.56^{a}	9.29^{ab}	11.38^{bc}	11.54^{c}
P2	12.18^{b}	9.13^{c}	11.06^{b}	11.06^{b}	11.38^{b}
P3	12.66^{c}	9.77^{d}	10.25^{b}	12.18^{c}	13.30^{d}
P4	12.98^{c}	12.34^{e}	8.81^{a}	12.82^{c}	10.41^{a}
P5	11.54^{b}	12.02^{e}	12.50^{c}	10.25^{ab}	10.58^{a}
P6	10.25^{a}	7.69^{b}	11.38^{bc}	9.45^{a}	11.22^{b}
P7	13.63^{d}	9.77^{d}	8.65^{a}	----------	----------
Average content	11.97	9.61	1027	11.19	1140
CV%	10.43	21.88	14.07	11.02	9.04

Averages with the same letters do not differ statistically at the level of 5% by the Duncan test, at the ambit of the native progenies of grass-mate.

It can be noticed signifficant differences at the average content of Mn at the progenies P1 (286.57) e P2 (234.20) comparing to the others progenies. According to Reissmann and Carneiro (2004) it is possible that one progenie be a Mn-tolerant and can accumulate this mineral, because it presents very high contents, above 1000 mg Kg^{-1} M.S., when compared to others progenies, without present toxicity signals. At the case of Zn the progenies P6 presented the highest concentration with 3.20 mg kg^{-1}, being the general average 2.51 mg kg^{-1}. It can be residue. At the present paper the values obtained used the thermo gravimetric analysis, with better sensibility. Among the compounds of great nutritional and pharmacological interest at the physic-chemical composition of grass-mate (*Ilex paraguariensis* St. Hil.) is the caffeine. According to Brenelli (2003) caffeine is an alkaloid with biological activity (Fig.1), containing nitrogen, with varied pharmacological action; among other effects, caffeine causes alteration at the cardiovascular and central nervous system. Caffeine belongs to

a class of natural occurring compounds called xantin, that possibly, are the most ancient stimulant known, and at this context caffeine is one of the most potent.

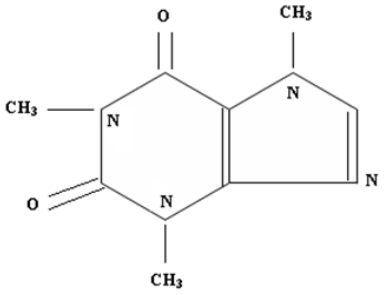

Fig. 5. Chemical structure of caffeine

Table 2 presents the average contents of caffeine (mg/g) found at the samples of native grass-mate, cultivated at the indigenous reserve of Kaiowá and Guarani, District of Caarapó-MS. It can be seen that the average contents of caffeine (6.56 mg/g a 13.63 mg/g), for this study with young leaves, are similar with the range presented at the papers of Esmelindro *et al*. 2002 and Bastos *et al*. 2006. Giberti (1979) describes that depending on the specie, can happen variation at the taste, size and texture of the leaves and at the color of the hastes, varying the intensity of their properties. Data from Instituto Adolfo Lutz (1962), obtained with 100g of grass-mate showed many substances at its composition, and specifically about caffeine the concentration was varying from 0.8 to 2.0g/100g. According to Reissmann *et al*. (1999) the qualitative and quantitative determinations, can not be considered definitive, mainly because they present genetic interferences and characteristics of the different environmental. Buhrer (2001) describes that the natural factors can interfere directly at the composition and physic-chemical properties of the grass-mate, and can determinate the final quality of the product and its organoleptic characteristics.

Conclusions

- It is important to emphasize that the selection of genetic material from a specific region with natural occurrence of a certain specie can conduct to the formation of an improved populations; these populations with have good level of productivity and broad genetic variation.
- The TG-DTG and DSC curves showed that the stability and thermal decomposition of the progenies can vary at the same ranges; it is not observed significant changes at the samples thermo decompositions, because the characteristics events of the minerals are preserved.

The physic chemical composition of the macro and micronutrients were find at the sufficient level for the culture, as far as the caffeine content that was considered satisfactory, showing that the program of genetic improvement can reflect significantly to improve the native grass-mate progenies quality at the indigenous reserve of Kaiowá/Guarani.

Aknowlegments

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