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CHARACTERIZATION OF FOLIAR SECONDARY METABOLITES IN BUTIA YATAY (MART.) BECC.

CARACTERIZAÇÃO DE METABÓLITOS SECUNDÁRIOS FOLIARES EM *BUTIA YATAY* (MART.) BECC.

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Abstract: *The palm tree Butia yatay (Mart.) Becc. occurs naturally in the municipality of Giruá, of the state of Rio Grande do Sul. Currently, it is in population decline, due to extensive agriculture and livestock activities that are expanding. In response to ecological pressures, many secondary compounds are synthesized by plants which perform several ecological functions. The main aim of this study was to characterize the foliar secondary metabolites of Butia yatay. Ten samples of the leaves of the species were collected, five of which were selected in the urban zone and five in the countryside of the municipality of Giruá. With foliar extracts, the qualitative phytochemical characterization of the secondary metabolites was carried out by means of specific chromatic tests. It was verified the presence of four classes of secondary metabolites: coumarins, flavonoids, saponins, and tannins. These compounds are assigned important functions in plants against biotic and abiotic factors. Moreover, they are of great interest to human health, especially from a pharmacological point of view due to the description of their biological effects.*

Keywords: *Arecaceae. Butia. Phytochemical characterization. Ecological functions.*

Resumo: A palmeira *Butia yatay* (Mart.) Becc. ocorre naturalmente no município de Giruá, localizado no estado do Rio Grande do Sul. Atualmente, encontra-se em declínio populacional, devido às atividades de agricultura e pecuária extensiva que estão em plena expansão. Em resposta às pressões ecológicas, muitos compostos secundários são sintetizados pelas plantas, os quais desempenham várias funções ecológicas. O principal objetivo deste estudo foi caracterizar os metabólitos secundários foliares de *Butia yatay*. Foram coletadas dez amostras das folhas da espécie, sendo cinco selecionadas na zona urbana e cinco na zona rural do município de Giruá. A partir dos extratos foliares, realizou-se a caracterização fitoquímica qualitativa dos metabólitos secundários por meio de ensaios cromáticos específicos. Constatou-se a presença de quatro classes de metabólitos secundários: cumarinas, flavonoides, saponinas e taninos. A estes compostos são atribuídas importantes funções nas plantas contra fatores bióticos e abióticos. Além disso, são de grande interesse para a saúde humana, sobretudo do ponto de vista farmacológico devido a descrição de seus efeitos biológicos.

Palavras-chaves: Arecaceae. Butia. Caracterização fitoquímica. Função ecológica.

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1 INTRODUCTION

Through secondary metabolism biosynthesis pathways, three main groups of secondary metabolites are formed, also called special metabolites, which are chemically distinct from each other, being terpenes, phenolic compounds, and nitrogen compounds, which are specific to a plant species or to a group of related species (TAIZ & ZEIGER, 2009). Compounds derived from "secondary" pathways are vital to plants and play important ecological functions related to protection against herbivorous and pathogen attack, competition between plants, water stress, attraction of pollinating organisms, seed dispersers and symbiotic microorganisms, among others (TAIZ & ZEIGER, 2009). Thus, the chemical diversity allied mainly to the phenotypic plasticity of the plants help to mediate biotic and abiotic factors (BUCHANAN et al., 2015).

With the advancement of research in organic chemistry between the XIX and XX centuries, several substances of secondary metabolism called plant active principles, now have biological application, through the recognition of their properties, mainly medicinal and pharmaceutical, with great commercial value from the economic point of view (SIMÕES et al., 2010). However, the great part of the plant species, especially the one present in the Brazilian territory, has never been analyzed chemically and pharmacologically and therefore, the conservation of areas with native species becomes an extremely important subject since these places can shelter a vast pharmacological potential still unexplored (GURIB-FAKIM, 2006).

The palm trees (Arecaceae) represent the third most important botanical family for humans (JOHNSON, 1998; ZAMBRANA et al., 2007). Except in Antarctica, Arecaceae has a worldwide distribution, with wide abundance, productivity and diversity of uses, being cultivated for ornamental purposes in urban arborization, in production food, medicines, and of great importance socio-cultural and economic for local populations (KINUPP & LORENZI, 2014). The Brazil has 438 species in 42 genera of palm trees distributed from southern Bahia to Rio Grande do Sul (BONDAR, 1964). The state of Rio Grande do Sul has six genera of native palm tree occur: *Bactris*, *Butia*, *Euterpe*, *Geonoma*, *Syagrus*, and *Trithrinax* (CORRÊA et al., 2009).

The genus *Butia* (Becc.) Becc. it belongs to the Butiinae subtribe, with some species occurring in regions of Argentina, Paraguay, and Uruguay. In Brazil, some species are found in the Midwest and Southeast of the country (CORRÊA et al., 2009; KINUPP & LORENZI, 2014). However, according to Leitman et al. (2014), the south of the country is the region that stands out most for the presence of this genera. The genus *Butia* is characterized by plant monoecious, protandric, and allogamous (CORRÊA et al., 2009). Most of the species belonging to this genus are known to form island populations called, “butiazais”, and do not occur in any other state of Brazil (SOARES et al., 2014). However, this genus is in declining population in the state of Rio Grande do Sul, due to extensive agriculture and livestock activities that are expanding in their areas of occurrence (SOARES & WITECK NETO, 2009).

In Southern Brazil, eight species belonging to the genus *Butia* were described, and the most prevalent species are *Butia yatay* (Mart.) Becc. (HOFFMANN et al., 2016), also known popularly as “butiá-jataí”, which has a wide natural distribution in Argentina and Uruguay (LORENZI et al., 2004; LORENZI, 2010). In Brazil, this species occurs in localities in the state of Rio Grande do Sul, among them, in the municipality of Giruá, as for example, in the “vale dos butiazais”, where only some individuals remain of what was a large “palmar” destroyed in the years 1970 for expansion of agricultural areas (MATTOS, 1977; SOARES et al., 2014). *Butia yatay* is a simple (solitary) thick, single palm tree that can reach up to 8m in height. The leaves are arched, greyish greens with rigid fibers, up to 3m in length. It presents branched interfoliate inflorescence, with ovoid, yellow or red fruits from 3.0 to 4.2cm in length, with succulent and bittersweet pulp (KINUPP & LORENZI, 2014).

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The research on palm trees of the genus *Butia* in southern Brazil is limited to the phytochemical, morphological and molecular characterization of the fruits of the "butiazeiros" (SOARES & WITECK NETO, 2009), and characterization studies of other parts of this plant such as leaves, not yet have been described, neglecting potentially unexplored phytochemical components. The aimed this study was to characterize the secondary foliar metabolites of the species *Butia yatay* (Mart.) Becc. through specific of chromatic tests in order to contribute with information already described for the species of the genus *Butia*, which are native and of great value, mainly, economic for the local communities of the state of Rio Grande do Sul.

2 MATERIALS AND METHODS

2.1 Study object

The study was carried out in the municipality of Giruá, which belongs to the North-West Mesoregion of Rio Grande do Sul (figure 1A) and to the Santo Ângelo Microregion (figure 1B), located at the intersection of 28°1'40"south latitude and 54°21'00"west longitude. With a territorial extension of 855.923 km² and 17.132 inhabitants, it is considered the agricultural productivity capital, a reference in the state and in Brazil for soy, wheat, canola, sunflower, and other crops, besides dairy potential. There are 420 m above sea level, its vegetation comprises a good part of fragments of the Atlantic Forest biome. It presents a humid subtropical climate, characteristic soil of neossolo and latossolo, with average annual temperature of 20 °C and annual average rainfall of 170 mm (IBGE, 2013; PREFEITURA MUNICIPAL DE GIRUÁ, 2015).

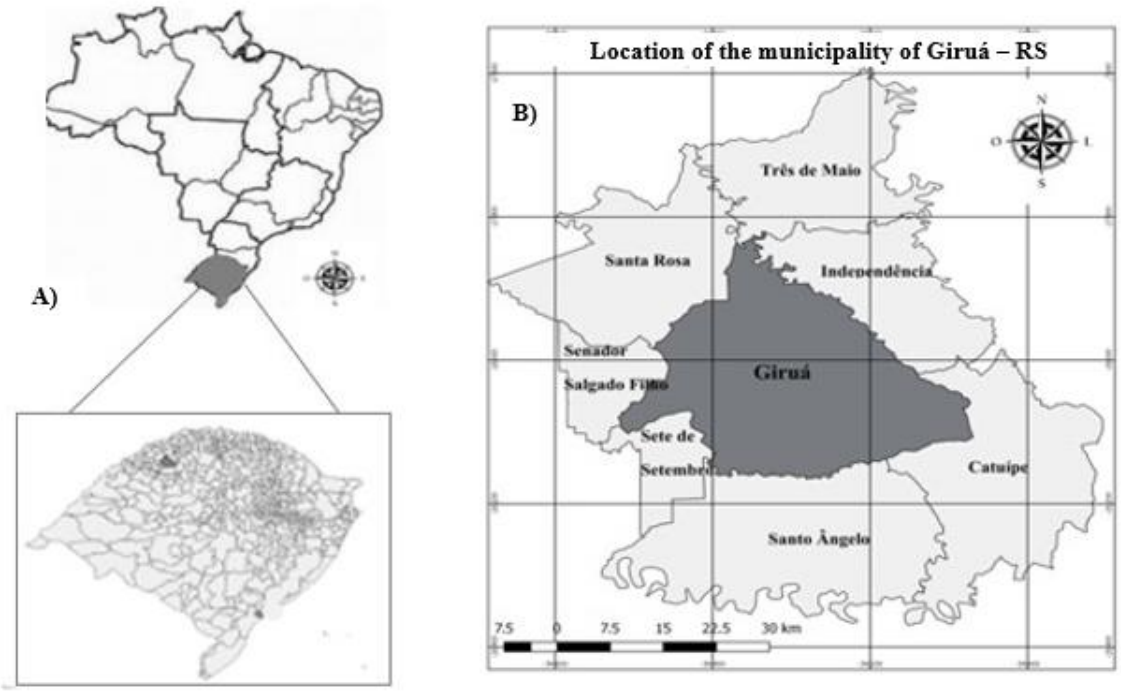

Figure 1. Study Area. A) Location of the municipality of Giruá in the Northwest Mesoregion of the State of Rio Grande do Sul – Brazil. B) Municipality of Giruá located in the Microregion of Santo Ângelo. Source: Own authorship (2019)

2.2 Action-research structuring

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In the municipality of Giruá, from September to October 2016, the samples were selected in different geographic positions, so that they were well represented in both urban zone and countryside. The monthly temperature averages for the sampling months corresponded to the minimum of 10.1°C and 14.9°C with maxims of 23.1°C e 25.9°C and rainfall between 52.4 mm and 299.8 mm, respectively (IRGA, 2016). The collection was carried out together with the Empresa de Assistência Técnica e Extensão Rural – EMATER/RS, of the Giruá unit.

Ten samples of the *Butia yatay* (Mart.) Becc. leaves were collected, and five samples were selected at two different points in the urban zone and countryside. In countryside, the samples were collected in the "vale dos butiazais" (private property) 6 km from the municipality, and in the vicinity of the highway RS 344 that gives access to the city. In the urban zone, the samples were extracted in the central region of the municipality. To obtain a pattern of the samples, the leaves of the median portion of the plant were chosen and those that were visually healthy. The specie exsiccates were stored at the Herbarium of the Universidade Regional Integrada do Alto Uruguai e das Missões, URI Campus Santo Ângelo – RS.

The collected material was sent to the pharmacognosy laboratory of the University, URI Campus Santo Ângelo – RS. From the pulverized plant material and extract production, the qualitative phytochemical characterization of eight main secondary metabolites was obtained, inferring its presence or absence, by means of staining and precipitation methods through specific methodology for each of the characterized compounds (table 1).

Table 1. Methods used for phytochemical characterization of leaf secondary metabolites in *Butia yatay* (Mart.) Becc.

Phytochemical Analysis		
Secondary metabolites	**Methodology used***	**References**
Volatile oils	Extraction on Clevenger apparatus	Farm. Bras. IV (1988)
Flavonoids	Shinoda reactions, Alkali hydroxides and UV fluorescence	Simões et al. (2004)
Alkaloids	Mayer, Dragendorff, and Bouchardat reagentes	Costa (2002)
Cardiotonic glycosides	Kedde reactions, Baljet, Leibermann-Burchard, and Keller-Kiliani	Macek et al. (1972)
Coumarins	UV fluorescence	Costa (2002)
Saponins	Determination of foam index	Farm. Bras. IV (1988)
Tannins	Irons salts reactions and lead acetate	Simões et al. (2004)
Cyanogenic glycosides	Test with pricoso paper	Costa (2002)

***** The methods for phytochemical characterization proposed by the authors, cited in the table above, were adapted as necessary. Source: Own authorship (2019)

3 RESULTS AND DISCUSSIONS

From the phytochemical characterization tests used in this study, in all samples, four classes of secondary metabolites from the eight analyzed were found in the leave extracts of *Butia yatay* (Mart.) Becc.: coumarins, flavonoids, saponins, and tannins (table 2).

Table 2. Qualitative characterization of the secondary metabolites of *Butia yatay* (Mart.) Becc.

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Phytochemical Analysis		
Secondary metabolites	**Methodology used***	**Results**
Volatile oils	Extraction on Clevenger apparatus	Absence (in 100g plant material)
Flavonoids	Shinoda reactions, Alkali hydroxides and UV fluorescence	Present
Alkaloids	Mayer, Dragendorff, and Bouchardat reagentes	Absence
Cardiotonic glycosides	Kedde reactions, Baljet, Leibermann-Burchard, and Keller-Kiliani	Absence
Coumarins	UV fluorescence	Present
Saponins	Determination of foam index	Present
Tannins	Irons salts reactions and lead acetate	Present
Cyanogenic glycosides	Test with pricoso paper	Absence

Source: Own authorship (2019)

Coumarins, flavonoids, and tannins are classes of compounds belonging to the phenolic compounds, which are characterized by having at least one phenol group in their structure, that is a functional hydroxyl group attached to an aromatic ring. Phenolic compounds are biosynthesized in plants by two pathways of secondary metabolism: malonic acid and the shikimic acid pathways, which is why they constitute a very heterogeneous group from the metabolic point of view (DEWICK, 2009; TAIZ & ZEIGER, 2009).

The presence of coumarins was verified by the intensification of the greenish-yellow coloration when submitted to UV light, indicating the positivity for this metabolite. In plants, coumarins can be synthesized in response to the attack of microorganisms and herbivores, and inhibit the germination of seeds of adjacent plants, preventing their competition for the same resources (allelopathy) (TAIZ & ZEIGER, 2009). According to Souza (2005), in human health coumarins play the role of defense of the organism and are found in vegetables, fruits, green tea among others. They are widely used by the industry as insecticides, food flavorings, medicines and cosmetics. Its numerous medicinal properties comprise biological activities such as, antiprotozoal, antidepressant, memory stimulant, anticoagulant, antioxidant, etc.

The presence of flavonoids was also confirmed by the reactions of Shinoda and alkaline hydroxides and by UV fluorescence with aluminum chloride solution (5%). According to Simões et al. (2004), it is possible to distinguish flavonoids from color observation by means of color tests. Thus, flavonic derivatives that are yellow, are reduced to reddish color, as verified in this study from the Shinoda reaction, indicating the presence of flavonols. In the reaction of alkaline hydroxides, the coloration obtained was yellowish-brown, this result can be correlated with the possible existence of chalcones. And, by fluorescence in the UV, with the addition of 5% aluminum chloride, it was observed the intensification of the greenish-yellow color, being able to be related to the presence of flavones. These results corroborate with studies that also report the presence of flavonols and flavones in leaf extracts obtained from palm trees (MACEK, 1972; WILLIAMS et al., 1985)

Flavonoids are assigned important physiological and ecological functions for plants, such as defense and pigmentation (SIMÕES et al., 2010). Besides, these secondary metabolites also stand out in the field of pharmacology due to their biological effects such as, antioxidants, antimicrobial, anti-inflammatory, antitumor, antiviral, among others (AMMAR et al., 2014; CRUXEN et al., 2016).

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The presence of tannins was detected due to the appearance of the green coloration without precipitate, through the reaction with the ferric chloride to 2%, indicating the positivity for condensed tannins. However, for hydrolyzable tannins, it was not possible to observe the presence of whitish precipitate, when 10% lead acetate was added, indicating the absence of this secondary metabolite, confirming the characteristics reported by Veiga Junior et al. (2005). The condensed tannins are compounds formed by the polymerization of flavonoid units. In plants, these compounds are important in the defense and protection of plants against herbivory (TAIZ & ZEIGER, 2009).

In general, humans prefer a certain level of astringency in foods containing tannins, such as apples, blackberries, tea, and grapes (TAIZ & ZEIGER, 2009). Moreover, according to Loguercio (2005), many physiological human activities have been attributed to the tannins, such as stimulation of phagocytic cells and tumor action, and anti-infective and antioxidant activities, antimicrobial properties among others.

The assays were also positive for saponins, this class of secondary metabolites belong to the terpene group. Terpenes are synthesized from the mevalonic acid (MEV) pathways in the cytoplasm and from methylerythritol phosphate (MEP) in chloroplasts. All terpenes are derived from pentacarbonated units (C5), isoprene. The variation in the amount of isoprenes is used for the classification of terpenes. In the case of saponins, these are classified as glycosides triterpenes (C30) so called due to their detergent and emulsifying action by these lipophilic (steroids or triterpenes) and hydrophilic (sugars) elements, when present in the same molecule, conferring the saponin when mixed with water (TAIZ & ZEIGER, 2009).

In this sense, the positivity for saponins was only confirmed qualitatively by the persistence of the foam, after fifteen minutes with thickness corresponding to 1cm. According to Simões et al. (2004), the presence of saponins in a plant drug is detected by the physical characteristic of abundant and persistent foam formation, with a foam column of at least 1cm remaining, for at least 15 minutes. Saponins act on plants to protect against insects and pathogens, as well as maintaining. Among the many activities of saponins investigated by human medicine, these are important for the action of vegetal drugs, used as expectorants and diuretics (TAIZ & ZEIGER, 2009).

Therefore, it is possible that these classes of secondary metabolites found in the leaves of *Butia yatay* (Mart.) Becc, are of great importance for the defense and protection of the plants, being the study of these substances fundamental to understand how the plants respond against biotic and abiotic factors, as described above. In addition, these compounds may also be of great pharmaceutical importance and hence to human health with proven biological effects. Besides, the “butiazais” ecosystem is recognized for their landscape, historical-cultural, and biodiversity value (RIVAS & BARBIERI, 2014).

4 CONCLUSIONS

From the phytochemical analysis performed in this study, it was possible to observe the presence of four classes of secondary metabolites in the *Butia yatay* (Mart.) Becc extract: coumarins, flavonoids, and tannins, which belong to the group of the phenolic compounds, and the saponins, these being of the terpenes group. As described in this work, these compounds play essential functions in plants, as well as, are important, especially from a pharmacological point of view, due to their biological potential, and therefore, being of great interest to man. However, it is necessary to emphasize that the qualitative phytochemical characterization, by means of chromatic assays, is a preliminary analysis and it is necessary to develop complementary studies aiming at a more robust phytochemical analysis of the

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secondary metabolites found in this study Besides, this chemical characterization may also help in sustainable exploration studies of "butiazeiros" in the southern region of the country.

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