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Phytochemical Characterization and *In vitro* Effects of Extracts Produced from Different *Maytenus ilicifolia* Matrices on the Activity of Intestinal Disaccharidases

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MSZS, JDM, MPM and LZ designed the study. Authors MSZS, JDM, ALR and LZ performed the analysis and wrote the first draft of the manuscript. Authors CF, GA, JFFC and MSZS managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Maytenus ilicifolia* Mart. Ex Reiss, Celastraceae, popularly known as "espinheira-santa" is traditionally used to treat gastrointestinal disorders and diabetes. However, studies proving efficacy for the treatment of diabetes are scarce. Furthermore, it is believed that the

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presence of chemical constituents responsible for pharmacological activity may be affected by environmental variations. Thus, the objective of this research was to evaluate the occurrence of variations in chemical composition, total polyphenol content, total tannin, antioxidant and antidiabetic activity *in vitro* for different matrices of *M. ilicifolia*.

Methodology: Chemical characterization was determined by CG-MS. Total polyphenol and total tannin contents were determined by spectrophotometer readings using standard gallic acid and tannic acid curves, respectively. *In vitro* antioxidant potential was determined by reducing the DPPH radical. *In vitro* antidiabetic activity was determined by inhibiting intestinal disaccharidases (maltase, sucrase and lactase) from a commercial glucose measurement kit produced by incubating intestinal homogenates with their substrates.

Results and Discussion: The results indicated the presence of variations in the chemical constituents and their concentrations, the total polyphenol content, total tannins and the *in vitro* antioxidant activity among the different tested extracts of *M. ilicifolia*. It is believed that these variations may be responsible for the differences found in inhibition of disaccharidases for the three intestinal enzymes.

Conclusion: Extracts 116 and 122 showed the best results in disaccharidase inhibition, however further studies are needed to investigate the results and reproducibility *in vivo.*

Keywords: Maytenus ilicifolia; chemical characterization; antioxidant activity; intestinal disaccharidases.

1. INTRODUCTION

Growth, development and biosynthesis of secondary metabolites in plants are negatively affected by environmental factors [1]. Among the environmental factors also called abiotic factors, temperature, salinity, water stress, habitat, fertilizer variations, cultivation conditions, geographical location, harvesting methods and post-harvesting techniques (drying, extraction) are some of the possible factors responsible for the synthesis, accumulation and distribution of secondary metabolites [1,2].

Plants produce a wide variety of structurally complex chemical compounds that are classified into primary and secondary metabolites, which are responsible for plant defense against biotic and abiotic stresses [3]. In addition, these secondary metabolites are of high interest in pharmacology due to the various effects on the human biological system [3].

Maytenus ilicifolia Mart. Ex Reiss, part of the Celastraceae family, is known as espinheira-santa and has a popular use indicated for the treatment of gastric ulcers and gastritis [4]. Moreover, the species has been popularly referred as effective for diabetes mellitus (DM) control [5,6] however, without scientific evidence.

Some studies have shown that *M. ilicifolia* undergoes changes in the composition of its secondary metabolites due to environmental factors [7,8]. Among its main secondary metabolites, polyphenols, flavonoids, tannins and triterpenes stand out as responsible for the therapeutic effects presented by the plant [8]. Among these, triterpenes are highlighted because of their antidiabetic property through various mechanisms of action in the body [9].

The genus Maytenus has been described in the literature for its antioxidant activity [6,10,11]. Similarly, plants with antioxidant activity have high potential for the treatment of various pathologies such as DM [12–14].

It has been dated that the use of medicinal plants millennially for the treatment of various pathologies is effective, however some of these plants need to be pharmacologically evaluated to prove their effectiveness in controlling DM [15]. Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high concentrations of glucose in the bloodstream (hyperglycemia) [16] and which has been increasing significantly [17].

Among the various classes of antidiabetic drugs available on the market are α-glucosidase inhibitors. The α-glucosidase enzymes are located at the edge of intestinal cells and play a role in the hydrolysis of carbohydrates resulting from a diet in monosaccharides that can be absorbed into the intestinal mucosa. When inhibition of α-glucosidase enzymes occurs, the process of glucose absorption is impaired, leading to a reduction in the glycemic level, especially postprandial [18].

Currently, four α-glycosidase inhibitors are available in the pharmaceutical market for the treatment of DM: acarbose, miglitol, voglibose

and emiglitte, acarbose being the best known and most prescribed drug. Administration should be performed orally, with meals. However, α-glucosidase inhibitors have serious adverse effects such as flatulence, severe abdominal pain and diarrhea [19–21]. It is in this sense of minimizing adverse drug effects that research on natural products and medicinal plants that have antidiabetic activity has been significantly increasing [22].

Therefore, the aim of this study was to evaluate if there are variations in the chemical constituents and pharmacological properties of different *M. ilicifolia* accesses through the measuring of the total polyphenol content, total tannins and evaluation of antioxidant and antidiabetic activity *in vitro*.

2. MATERIALS AND METHODS

2.1 Plant Material

Seed collection from different matrices of *M. ilicifolia* occurred in different cities of Rio Grande do Sul, where each matrix gave rise to the denomination of access (Table 1). The seedlings produced from these seeds were then planted on the campus of the Federal Institute of Science and Technology of Rio Grande do Sul (Instituto Federal de Ciência e Tecnologia do Rio Grande do Sul) in 2006 (latitude 31° 42 '47,48868 "S and longitude 52° 18' 40,05201 "W), in the city of Pelotas/RS. For the present experiment the leaves of *M. ilicifolia* were collected in March 2017, shortly after the fruiting period, the leaves were subsequently dried in forced air at 40ºC, until constant weight and fragmented with the aid of a food shredder.

2.2 Preparation of Ethanolic Extracts

The extracts were obtained by the reactive solvent method from maceration, where 5 grams of each vegetable matrix were added in 45 mL of 98% ethyl alcohol, without agitation and without light. After five days the mixtures were filtered on filter paper, rotary evaporated, identified and stored in glass vials in a freezer at -20°C. The average yield of *M. ilicifolia* leaf extracts was approximately 9%.

2.3 Chromatographic Analysis and Chemical Identification

The chemical profile of the extracts was obtained by the High Performance Liquid Chromatography technique (HPLC) on a Shimadzu (LCMS-2020) chromatograph model SPD-M20A with PDA detector. Reverse phase analysis were conducted under gradient conditions with C18 column (4.6mm x 250mm) containing 5µm particle size particles [24]. The mobile phase applied in the procedure was a mixture of methanol and aqueous formic acid (5%). The concentration gradient was applied over 65 minutes at a flow rate of 0.6 ml/min as follows: first 5-15 for 10 minutes. Finally, the chromatographic-level chemical profile of the samples was compared to the NIST library (National Institute of Standards and Technology), coupled with the equipment's memory, as well as previous calibration with analytical standards (catechin, epicatechin, caffeic acid, rosmarinic acid, quercitrin, quercetin, rutin and kaempferol).

2.4 Determination of the Total Polyphenol Content

The total polyphenol content of the extracts was determined by the Folin - Ciocalteau method [25]. In 20 µL of extract, 150 µL of distilled water and 10 µL of Folin-Ciocalteau reagent in this order were added. The solution was mixed while standing for 3 minutes. Then 30 µl of saturated sodium carbonate solution was added, leaving the solution to stand in the dark for 1 hour. The blank solution was prepared under the same conditions by replacing the volume of the extract with the solvent contained in the extract. Analyzes were performed in triplicate. After time, the readings were taken in a spectrophotometer at a wavelength of 765 nm. Total polyphenol content was quantified based on the standard gallic acid curve with solutions ranging from 5 to 400 $mg \cdot L^{-1}$. Phenolic content was expressed in mg of gallic acid (EAG) per gram of extract.

Table 1. Seed collection sites from different matrices of *Maytenus ilicifolia*. Each array originated an access

Access	Provenance	Seed Collection date
116, 117, 118, 122	Canguçu	Jan/2003 and Nov/2003
123	Morro Redondo	Dec/2003
127, 129, 130, 131, 133, 135, 136	Piratini	Dec/2002 and Dec/2003
137	Pelotas	Dec/2002 and Dec/2004

Source: Perleberg, 2017 [23]

2.5 Determination of Tannin Content

The total tannin content of the extracts was determined by the Folin-Denis method [26]. In 50 µL of extract, 100 µL of distilled water, 50 µL of Folin-Denis reagent and 100 µL of saturated sodium carbonate solution were added. The solution was mixed and rested for 30 minutes. The blank solution was prepared under the same conditions by replacing the volume of the extract with the solvent contained in the extract. Analyzes were performed in triplicate. After time the readings were taken in a spectrophotometer at a wavelength of 760 nm. To quantify the total tannins in the extract, solutions ranging from 3 to 20 $mg \cdot L^{-1}$ of tannic acid were prepared. Total tannin content was expressed as a percentage of tannic acid per gram of extract.

2.6 Determination of the Antioxidant Potential by the DPPH Method

The antioxidant potential of the extracts was determined by the methodology where the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is reduced by products with antioxidant potential resulting in a violet to yellow color change proportional to the concentration of the reducing substance in the sample [27]. Ethanolic solutions of the samples were prepared at concentrations of 0.8 to 10 $\mu g \cdot L^{-1}$. To 213 µL of the solution was added 87 µL of a 0.3 mM DPPH solution.

As blank, 213 µL of solution and 87 µL of ethanol were used. As a control, 213 µL of ethanol and 87 µL of DPPH solution were used. The mixtures were kept dark for 30 minutes at room temperature for reaction between samples and DPPH to occur. After 30 minutes, the UV-Vis spectrophotometer was read at 517 nm. Analyzes were performed in triplicate. The determination of the percentage of antioxidant activity (AA) was made by the following formula:

$$A\ (\%) = 100 - \left[\frac{(Abs.sample - Abs.blank) * 100}{Abs.control}\right]$$

The graph was then plotted with the X-axis concentration and Y-axis antioxidant activity and the straight-line equation was plotted, where it was possible to find the extract concentration needed to reduce the initial amount of DPPH by 50%, thus determining the IC50.

2.7 *In vitro* Evaluation of the Inhibitory Activity on Intestinal Disaccharidases

For the evaluation of *in vitro* antidiabetic activity by inhibiting intestinal disaccharidases, adult male Wistar rats aged 45-55 days old, obtained from the Unochapecó bioterism center were used. After euthanasia of the animals a small bowel segment (10 cm) was removed, washed in 0.9% NaCl solution, dried on filter paper, weighed and homogenized with 0.9% NaCl (400 mg duodenum$\cdot mL^{-1}$), for 1 min at 4°C. The resulting homogenate was centrifuged at 8,000 rpm for 8 minutes and the supernatant was used for the evaluation of intestinal disaccharidase activity (maltase, sucrase and lactase) and for protein determination [28].

Firstly, the supernatant (10 µL) was incubated for 5 minutes at 37°C in the presence of one of the extracts of *M. ilicifolia* leaves in three different concentrations 250, 500 and 1000 $\mu g \cdot mL^{-1}$ [27], diluted in tween (*Polysorbate 80)*1% or with acarbose (positive control) at three different concentrations 20, 40 and 80 $\mu g \cdot mL^{-1}$ [29]. Then 10 µl of substrate (maltose, sucrose or lactose) was added and incubation was continued for 30 minutes at 37°C. After this time, 240 µL of glucose oxidase buffer was added and incubated for 10 minutes at 37°C. Subsequently, the spectrophotometer was read at a wavelength of 505 nm.

Proteins contained in the supernatant were quantified using bovine serum albumin [30] as standard and the assays performed in hexaplicate and with appropriate controls. Results were expressed as enzymatic activity per milligram of protein.

2.8 Statistical Analysis

Statistical comparisons were performed by two-way analysis of variance (ANOVA) followed by Bonferroni post-test. Results were expressed as mean ± standard error of the mean (SEM). Differences were considered significant when $p < 0.05$.

3. RESULTS AND DISCUSSION

From the analysis of the chemical constituents of the extracts by Liquid Chromatography, we identified the presence of epicatechin, rosmarinic acid, quercitrin and rutin in some of the *M. ilicifolia* accesses (Table 2).

Table 2. Concentration (mg·g^{-1}) of compounds identified in *Maytenus ilicifolia* accession extracts

Place	Access	Epicatechin	Rosmarinic Acid	Quercitrin	Rutin
Canguçu	116	81.3	10.0	16.3	16.6
	117	31.3	7.0	17.0	19.0
	118	103.0	-	11.0	12.0
	122	70.0	-	14.0	17.0
Morro Redondo	123	77.0	-	9.0	16.0
Piratini	127	-	7.0	11.0	6.0
	129	-	-	-	-
	130	-	-	10.0	9.0
	131	-	-	-	-
	133	-	-	16.0	-
	135	-	-	-	-
	136	-	-	-	-
Pelotas	137	-	-	-	-

Flavonoid-class compounds have been widely studied due to various biological activities, especially antidiabetic and antioxidant activity [31]. The results found for *M. ilicifolia* extracts are in agreement with studies already reported in the literature [6,32–34].

It is possible to notice that the extracts from the cities of Canguçu and Morro Redondo presented the highest concentrations of the constituent studied being epicatechin the major compound. For the extract from the city of Pelotas, the presence of any of the constituents surveyed was not found, reinforcing that environmental factors can change the content of secondary metabolites in plants.

The total polyphenol and total tannin contents quantified in the different extracts, as well as the *in vitro* antioxidant activity are presented in Table 3. The extracts of accesses 129, 118 and 136 presented the highest total polyphenol contents, representing 551.2; 514.9 and 501.7 mg of gallic acid per gram of extract, respectively. However, extracts obtained from accesses 130 and 133 presented the lowest total polyphenol contents, representing 273 and 325.7 mg of gallic acid per gram of extract, respectively.

The highest percentages of total tannins were found in extracts obtained from accessions 123, 131 and 130 representing 7.60; 7.54 and 7.37% respectively. The lowest percentages were found in accesses 136, 117 and 122 representing 5.51; 5.18 and 5.35% respectively.

The extracts obtained from accesses 129 and 130 presented the lowest concentrations needed to reduce the initial amount of DPPH by 50% representing 2.13 and 3.40 μg·mL^{-1}. However, extracts from accesses 122 and 131 presented the highest concentrations required to reduce the DPPH radical representing 8.62 and 8.55 μg·mL^{-1}.

Phenolic compounds are the most abundant secondary metabolites in plants and have several beneficial effects on various oxidative stress-associated diseases, such as cancer, Alzheimer's, diabetes, and cardiovascular disease [35]. Our results are in agreement with studies that indicate the presence of several chemical constituents of the family of polyphenols with biological activities for the genus Maytenus [6,36].

In addition, it is possible to establish a positive relationship with polyphenol content and antioxidant activity, as observed for accesses extract 129 and as also observed in other studies [37]. Moreover, the extracts with the lowest polyphenol contents belong to the same collection site, and it is possible to establish a relationship between the concentration of secondary metabolites and environmental conditions of cultivation.

The antioxidant potential of *M. ilicifolia* has been previously presented [38]. However, a study pointed out that the antioxidant activity can be directly influenced by the drying temperature of the leaves of *M. ilicifolia* [8].

Tannins are polyphenolic compounds present in various medicinal plants and food sources. Several studies indicate that tannins play an

important role in the prevention and management of diabetic complications such as nephropathy, neuropathy, retinopathy and diabetic cardiomyopathy [39].

Furthermore, studies have identified that the presence of several tannins may be possible chemical constituents responsible for the therapeutic effect of Maytenus species [40]. In addition, cultivars of *M. ilicifolia* exposed to high temperatures have higher concentrations of tannins as a defense mechanism against the incidence of ultraviolet rays [7].

In general, the results found are in accordance with studies present in the literature, where the authors observed that seasonal changes may be responsible for significant variations in the content of chemical constituents such as total polyphenols, flavonoids, tannins, reflecting significantly on antioxidant activity [41,42].

Based on the popular use of the genus Maytenus [4] for the treatment of diabetes and also in previous studies confirming this potential [43], the effect of extracts of different accesses of *M. ilicifolia* were evaluated in contrast to α-glucosidase enzymes. It was possible to verify that all the extracts showed action on the enzyme activity, some causing inhibition and others enzymatic stimulation.

Fig. 1 shows the percentages of maltase enzyme activity for the different extracts and acarbose in relation to the control. Extracts 116 and 123 in the three concentrations presented higher percentages of enzyme inhibition when compared to the control. For the concentration of 250 $\mu g \cdot mL^{-1}$ extracts 116 (65.59%) and 123 (64.53%) caused the highest inhibition percentage of the enzyme. In the concentration of 500 $\mu g \cdot mL^{-1}$ the highlight was the extracts 116 (70.32%) and 122 (72.17%). For the concentration of 1000 $\mu g \cdot mL^{-1}$ the extracts with the highest inhibition percentage were 116 (82.89%) and 137 (77.42%), however, extract 133 at this concentration was not able to cause enzyme inhibition. The inhibition percentages for acarbose averaged 65% for the different concentrations of 20, 40 and 80 $\mu g \cdot mL^{-1}$, thus indicating that some *M. ilicifolia* extracts have a similar degree of inhibition to the positive control (glycosidase inhibitor antidiabetic drug).

Table 3. Total polyphenols, total tannins and *in vitro* antioxidant potential of different *Maytenus ilicifolia* accessions

Place	Access/ Standard	Total polyphenols (mg GA/g extract)	Total tannins (%)	DPPH (IC_{50} – µg/mL)
Canguçu	116	398.8 ± 40.6	6.49 ± 0.07	5.92 ± 1.08
	117	434.4 ± 19.4	5.18 ± 0.18	5.81 ± 0.42
	118	514.9 ± 52.0	6.57 ± 0.53	4.99 ± 0.03
	122	343.3 ± 35.7 [c]	5.35 ± 0.42	8.62 ± 0.45 [abc]
Morro Redondo	123	469.1 ± 57.7	7.60 ± 0.53 [bd]	3.41 ± 0.14 [abcd]
Piratini	127	389.7 ± 80.5	5.79 ± 0.16 [e]	4.18 ± 0.33 [abd]
	129	551.2 ± 55.8 [adf]	4.79 ± 0.16 [ace]	2.13 ±0.40 [abcdf]
	130	273.0 ± 36.2 [bceg]	7.37 ± 0.99 [bdfg]	4.38 ± 0.44 [adg]
	131	406.5 ± 29.1 [g]	7.54 ± 0.17 [bdfg]	8.55 ± 0.16 [abcefghi]
	133	325.7 ± 18.6 [ceg]	6.06 ± 0.70 [e]	4.40 ± 0.81 [dgi]
	135	393.4 ± 47.5 [g]	6.65 ± 0.10 [bg]	3.40 ± 0.20 [abcdi]
	136	501.7 ± 33.0 [dhj]	4.51 ± 0.56 [acehijk]	5.80 ± 0.27 [defgik]
Pelotas	137	394.4 ± 16.1 [g]	6.47 ± 0.45 [gl]	4.67 ± 0.56 [dgi]
	Gallic Acid	-	-	2.62 ± 0.13 [abcdfhijlm]

Results are expressed as mean ± standard deviation (n=3). Statistical analysis was performed by one-way ANOVA followed by Bonferroni post-test, where [a]statistically different compared to the 116 group; [b]statistically different compared to the 117 group; [c] statistically different compared to the 118 group; [d]statistically different compared to the 122 group; [e] statistically different compared to the 123group; [f]statistically different compared to the 127 group; [g]statistically different compared to the 129 group; [h]statistically different compared to the 130 group; [i]statistically different compared to the 131 group; [j]statistically different compared to the 133 group; [k] statistically different compared to the 135 group; [l]statistically different compared to the 136 group; [m]statistically different compared to the 137 group. GA= gallic acid; DPPH= 2,2-diphenyl-1-picrylhydrazyl; IC_{50} = 50% inhibitory concentration

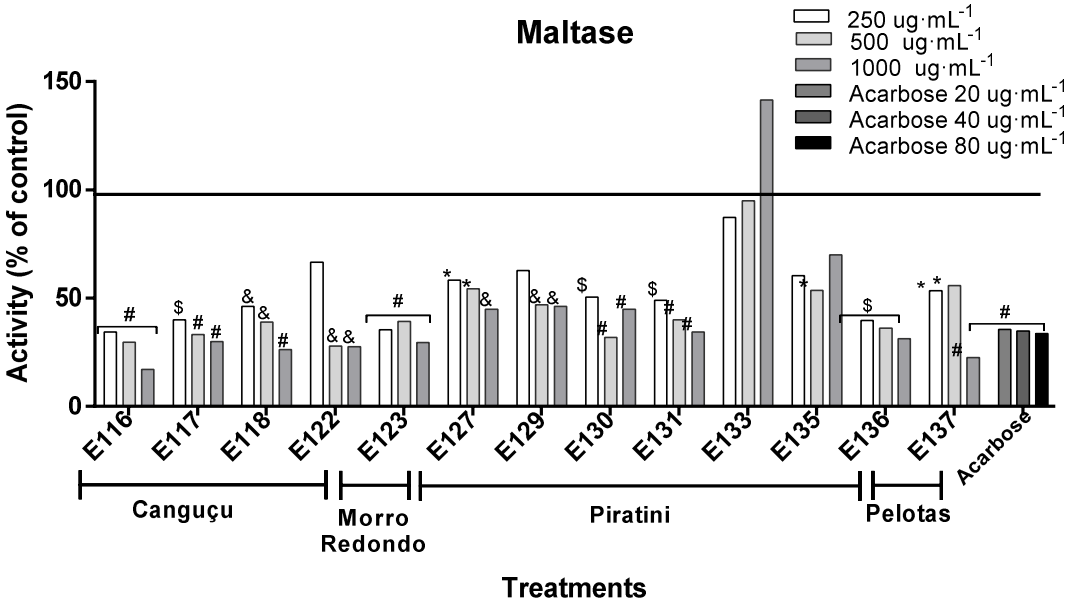

Fig. 1. Percentage of maltase enzyme activity after incubation with *M. ilicifolia* extracts at concentrations of 250, 500 and 1000 µg·mL^{-1} and acarbose at concentrations of 20, 40 and 80 µg·mL^{-1}

*Results were expressed as a percentage of the control representing the gut sample with 100% maltase activity (black time at the top of the image) and analyzed by two-way ANOVA followed by the Bonferroni post test. * p<0.05; & p<0.01; $ p<0.001; # p<0.0001 compared to control*

Fig. 2 shows the percentages of sucrase enzyme activity in the presence of different extracts and acarbose in relation to the control. Extracts 116, 122, 135, 136, 137 presented the highest percentages of enzyme inhibition when compared to the control group. For the concentration of 250 µg·mL^{-1} extracts 116 and 123 were responsible for the highest percentage of enzyme inhibition, representing 65.59% and 78.21%, respectively. Extracts 116 and 133 presented the highest inhibition percentage at the concentration of 500 µg·mL^{-1}, representing 70.32% and 75.51%, respectively. For the concentration of 1000 µg·mL^{-1} extracts 116 and 122 were responsible for the highest percentage of enzyme inhibition (82.90% and 78.10%). However, it was possible to realize that acarbose exhibited an enzyme inhibition superior to the effect of the extracts.

However, extracts 127 and 130 enhanced the enzyme activity at the three concentrations. Extract 131, in turn, stood out due to its high stimulating potential of the enzyme at a concentration of 250 µg·mL^{-1}.

Fig. 3 shows the percentages of lactase enzyme activity in the presence of the different extracts and acarbose in relation to the control. Extracts 116, 122, 135, 136, 137 were responsible for the decrease of enzyme activity when compared to the control group. At the concentration of 250 µg·mL^{-1}, extracts 127 and 135 were more prominent with enzyme inhibition percentage of 78.49% and 68.56%, respectively. For the concentration of 500 µg·mL^{-1}, the highlight was extracts 116 (70.32% inhibition) and 135 (74.17% inhibition). The inhibition percentage at the concentration of 1000 µg·mL^{-1} was more significant with extracts 116 and 133, representing 82.89% and 80.83%, respectively.

However, extract 118 did not cause enzyme inhibition at any of the concentrations tested, as did acarbose. Drugs belonging to the class of α-glycosidase inhibitors such as acarbose have no effect on this enzyme because disaccharide lactose does not contain α-glucosidase but β-glycosidase bonds. In case of inhibition of this enzyme the organism presents deficiency of digestive enzyme causing lactose intolerance [44].

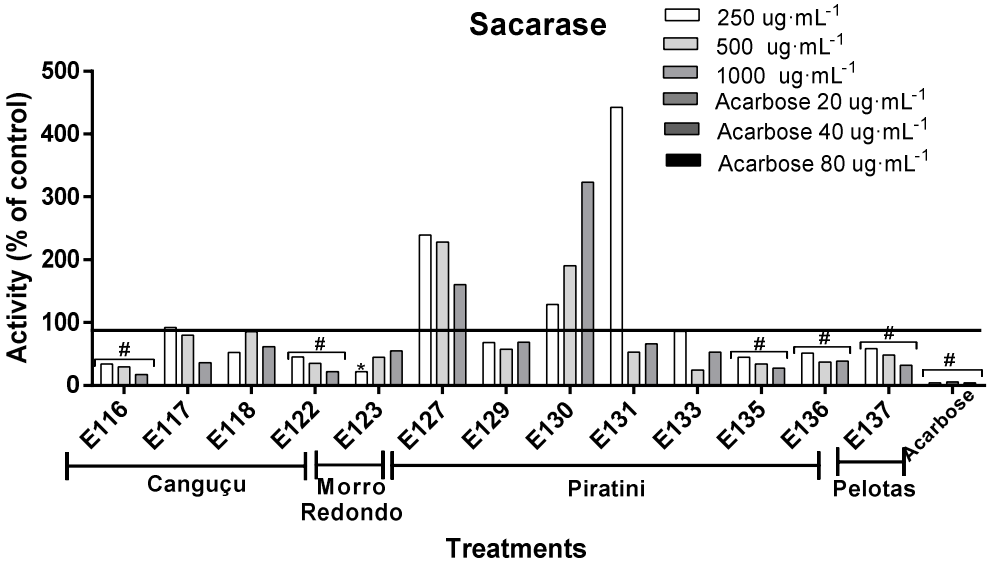

Fig. 2. Percentage of sucrase enzyme activity after incubation with *M. ilicifolia* extracts at concentrations of 250, 500 and 1000 µg·mL^{-1} and acarbose at concentrations of 20, 40 and 80 µg·mL^{-1}

*Results were expressed as a percentage of the control representing the gut sample with 100% maltase activity (black time at the top of the image) and analyzed by two-way ANOVA followed by the Bonferroni post test. * p<0.05; & p<0.01; $ p<0.001; # p<0.0001 compared to control*

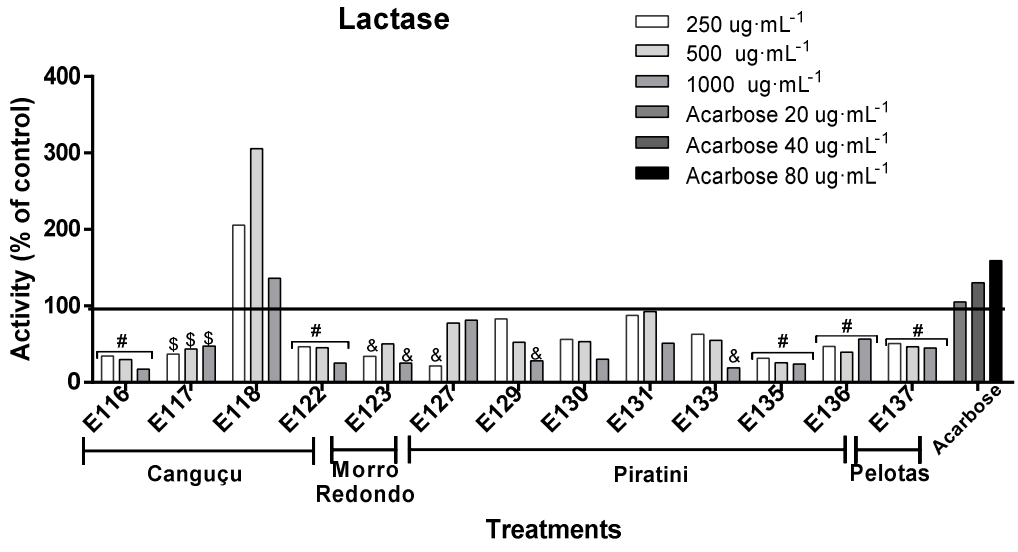

Fig. 3. Percentage of lactase enzyme activity after incubation with *M. ilicifolia* extracts at concentrations of 250, 500 and 1000 µg·mL^{-1} and acarbose at concentrations of 20, 40 and 80 µg·mL^{-1}

*Results were expressed as a percentage of the control representing the gut sample with 100% maltase activity (black time at the top of the image) and analyzed by two-way ANOVA followed by the Bonferroni post test. * p<0.05; & p<0.01; $ p<0.001; # p<0.0001 compared to control*

Several plants are popularly used for diabetes control worldwide and there are several mechanisms of action responsible for this effect, such as inhibition of α-amylase and α-glycosidase enzymes, as well as antioxidant activity [45]. In addition, several chemical constituents present in plants have potent inhibitory activity of α-glucosidase enzymes, among them terpenes, alkaloids, quinines, flavonoids, phenols and phenylpropanoids [46].

Multiple studies with medicinal plants and dietary fruits such as *Hibiscus sabdariffa* (popularly known as caruru azedo), *Phaseolus vulgaris* (popularly known as feijão-de-trepa), *Antidesma bunius* (popularly known as bignay), some species of algae, rowanberry and raspberry, indicated the presence of bioactive compounds with antioxidant and antidiabetic activity due to inhibition of intestinal enzymes α-amylase and α-glucosidase [36,47].

Studies have shown that pomegranate peel (*Punica granatum* L.) ethanolic extract, coffee extract, apple juice and grape marc (byproduct generated by the wine industry) caused inhibition of α-glycosidases enzymes, delaying digestion of carbohydrates and consequently lowering the glycemic level. Thus, it is possible to establish a positive relationship between natural products and glycosidase inhibitory activity applied in the treatment of type 2 DM [48–52].

However, in our study it was possible to observe differences in the chemical constituents and their concentrations, in addition to the contents of total polyphenols, total tannins, the antioxidant and antidiabetic activities of the different *M. ilicifolia* accessions. Considering that each access was obtained from (not identical) sister plants, environmental factors and genetic profile may be responsible for the discrepancy of the results.

Some studies indicate that environmental changes (altitude, rainfall, macro and micronutrients in the soil, relative humidity, temperature, climate, soil pH) significantly affect the morphological and morphoanatomic characteristics of the plants, especially the *M. ilicifolia* species, directly affecting the concentration of secondary metabolites and consequently the biological effects exerted [7,20,53–55]. In addition, the harmful effects caused by environmental contamination affect plant physiological, biochemical and antioxidant processes, affecting the quality of natural products [56].

4. CONCLUSION

Through this study, it was possible to realize that extracts of different matrices of *M. ilicifolia* presented variations in the chemical constituents and their concentrations. In addition, differences in total polyphenols, total tannins, antioxidant and glycosidase inhibitor activities were observed, and environmental factors may be responsible for this discrepancy in the results. However, extracts from accesses 116 and 122 showed promising results in inhibiting α-glucosidase enzymes *in vitro*, and therefore we emphasize the need for further studies to investigate the antidiabetic potential of the species *in vivo*.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

The experimental protocol was approved by the Animal Use Ethics Committee of the Community University of Chapecó Region - Unochapecó (CEUA 004/2017).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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