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

Monica S. Z. Schindler¹, Carine Frozza², Gabriela Anzollin², Jean F. F. Calisto³, André L. Radünz⁴, Márcio P. Mariot², Jacir Dal Magro¹,³ and Leila Zanatta¹,⁶*

¹Graduate Program in Environmental Sciences, Community University of the Region of Chapecó – Unochapecó, Brazil.
²Pharmacy Course, Community University of Chapecó Region – Unochapecó, Brazil.
³Chemical Engineering Course, Community University of Chapecó Region – Unochapecó, Brazil.
⁴Agronomy Course, Federal University of Fronteira Sul (UFFS), Brazil.
⁵Agronomy Course, Federal Institute of Science and Technology of Rio Grande do Sul (IFSuS), Brazil.
⁶Western Higher Education Center, Santa Catarina State University – UDESC, Brazil.

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.

Article Information

DOI: 10.9734/EJMP/2020/v31i1430315

Editors:
(1) Dr. N. Karmegam, Government Arts College, India.
(2) Marcello Iriti, University of Milan, Italy.

Reviewers:
(1) Masheer Ahmed Khan, Devi Ahilya Vishwavidyalaya, India.
(2) Santosh Kanaji, BLDEA’s SSM College of Pharmacy, India.
(3) Jackson Godwin, Niger Delta University, Nigeria.

Complete Peer review History: http://www.sciarticle4.com/review-history/60984

Received 05 July 2020
Accepted 10 September 2020
Published 22 September 2020

ABSTRACT

Introduction: Maytenus ilicifolia Mart. Ex Reiss, Celastraceae, popularly known as “espinheirasanta” 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
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:** Exacts 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].

*M. 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 millenially 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 emiglottite, 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 -20ºC. 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·L⁻¹. Phenolic content was expressed in mg of gallic acid (EAG) per gram of extract.

| 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·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 µg·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:

$$A (%) = 100 - \left( \frac{(A_{\text{sample}} - A_{\text{blank}}) \times 100}{A_{\text{control}}} \right)$$

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·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 µg·mL$^{-1}$ [27], diluted in tween (Polysorbate 80)1% or with acarbose (positive control) at three different concentrations 20, 40 and 80 µg·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).
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 accessions 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

### 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    | -           | -               | -          | -     |
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 accessions 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 μg·mL\(^{-1}\) extracts 116 (65.59%) and 123 (64.53%) caused the highest inhibition percentage of the enzyme. In the concentration of 500 μg·mL\(^{-1}\) the highlight was the extracts 116 (70.32%) and 122 (72.17%). For the concentration of 1000 μg·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 μg·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                       | 5.35 ± 0.42       | 8.62 ± 0.45                 |
| Morro Redondo| 123             | 469.1 ± 57.7                       | 7.60 ± 0.53       | 3.41 ± 0.14                 |
| Piratini     | 127             | 389.7 ± 80.5                       | 5.79 ± 0.16       | 4.18 ± 0.33                 |
|              | 129             | 551.2 ± 55.8                       | 4.79 ± 0.16       | 2.13 ± 0.40                 |
|              | 130             | 273.0 ± 36.2                       | 7.37 ± 0.99       | 4.38 ± 0.44                 |
|              | 131             | 406.5 ± 29.1                       | 7.54 ± 0.17       | 8.55 ± 0.16                 |
|              | 133             | 325.7 ± 18.6                       | 6.06 ± 0.70       | 4.40 ± 0.81                 |
|              | 135             | 393.4 ± 47.5                       | 6.65 ± 0.10       | 3.40 ± 0.20                 |
|              | 136             | 501.7 ± 33.0                       | 4.51 ± 0.56       | 5.80 ± 0.27                 |
| Pelotas      | 137             | 394.4 ± 16.1                       | 6.47 ± 0.45       | 4.67 ± 0.56                 |
| Gallic Acid  | -               | -                                  | -                 | 2.62 ± 0.13                 |

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 123 group; ‘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-glucosidase 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).

ACKNOWLEDGEMENTS

This work was supported by the CAPES/PROSUP 2017 and Unochapecó.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. AshrafMA, IqbalM, RasheedR, HussainI, RiazM, ArifMS. Environmental stress and secondary metabolites in plants, in: Plant metabolites and regulation under environmental stress. Elsevier. 2018:153–167. Available:https://doi.org/10.1016/B978-0-12-812689-9.00008-X

2. MoghaddamM, Meh dizadehL. Chemistry of essential oils and factors influencing their constituents, in: Soft chemistry and food fermentation. Elsevier. 2017:379–419.
3. Wang S, Alseekh S, Fernie AR, Luo J. The structure and function of major plant metabolite modifications. Molecular Plant. 2019;12:899–919. Available: https://doi.org/10.1016/j.molp.2019.06.001

4. Almeida C, Barbieri RL, Ribeiro MV, Lopes CV, Heck RM. Espinheira-santa (Maytenus ilicifolia Mart. ex Reiss.): Saber de erva e feirantes em Pelotas (RS), Revista Brasileira de Plantas Medicinais. 2015;17:722–729. Available: https://doi.org/10.1590/1983-084X/14_003

5. Mariot MP, Barbieri RL. O Conhecimento popular associado ao Uso da Espinheira-santa (Maytenus ilicifolia e M. aquifolium), Revista Brasileira de Biociências. 2008;5:666–668.

6. Veloso CC, Soares GL, Perez AC, Rodrigues VG, Silva FC. Pharmacological potential of Maytenus species and isolated constituents, especially tingenone, for treatment of painfull inflammatory diseases, Revista Brasileira de Farmacognosia. 2017;27:533–540. Available: https://doi.org/10.1016/j.bjp.2017.02.006

7. Missi A, Mazutti M, Parouil N, Corazza M, Dariva C, Cansian RI, Oliveira JV. Chemical variation of tannins and triterpenes in Brazilian populations of Maytenus ilicifolia Mart. ex Reiss, Brazilian Journal of Biology. 2009;69:339–345. Available: https://doi.org/10.1590/S1519-6984200900200015

8. Negril MLS, Possamaia JC, Nakashima T. Atividade antioxidante das folhas de espinheira-santa - Maytenus ilicifolia Mart. ex Reiss., seca em diferentes temperaturas. Revista Brasileira de Farmacognosia. 2009;19:553–556. Available: https://doi.org/10.1590/S0102-695X2009000400007

9. Nazaruk J, Borzym-Kluczyk M. The role of triterpenes in the management of diabetes mellitus and its complications. Phytochemistry Reviews. 2015;14:675–690. Available: https://doi.org/10.1007/s11101-014-9369-x

10. Bruni R, Rossi D, Muzzoli M, Romagnoli C, Paganello G, Besco E, Choquecillo F, Peralta K, Lora WS, Sacchetti G. Antimutagenic, antioxidant and antimicrobial properties of Maytenus krukovii bark, Fitoterapia. 2006;77:538–545. Available: https://doi.org/10.1016/j.fitoter.2006.06.009

11. Thiesen LC, da Silva LM, Santin JR, Bresolin TM, de Andrade SF, de MC, Amorim L. Merlin, R.A. de Freitas, R. Niero, D.J.A. Netz. Hepatoprotective effect of Maytenus robusta Reiss extract on CCl4-induced hepatotoxicity in mice and HepG2 cells. Regulatory Toxicology and Pharmacology. 2017;86:93–100. Available: https://doi.org/10.1016/j.yrtph.2017.02.023

12. Aboonabi A, Singhi T. The effectiveness of antioxidant therapy in aspirin resistance, diabetes population for prevention of thrombosis. Biomedicine & Pharmacotherapy. 2016;83:277–282. Available: https://doi.org/10.1016/j.biopha.2016.06.044

13. Pokorski M, Pozdzik M, Mazzatenta A. Antioxidant treatment for impaired hypoxic ventilatory responses in experimental diabetes in the rat. Respiratory Physiology & Neurobiology. 2018;255:30–38. Available: https://doi.org/10.1016/j.resp.2018.05.005

14. Sedaghat A, Shahbazian H, Rezazadeh A, Haidari F, Jahanshahi A, Mahmoud Latifi S, Shirbeigi E. The effect of soy nut on serum total antioxidant, endothelial function and cardiovascular risk factors in patients with type 2 diabetes. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2019;13:1387–1391. Available: https://doi.org/10.1016/j.dsx.2019.01.057

15. Xu L, Li Y, Dai Y, Peng J. Natural products for the treatment of type 2 diabetes mellitus: Pharmacology and mechanisms. Pharmacological Research. 2018;130:451–465. Available: https://doi.org/10.1016/j.phrs.2018.01.015

16. Wasserman DH, Wang TJ, Brown NJ. The Vasculature in prediabetes. Circ Res. 2018;122:1135–1150. Available: https://doi.org/10.1161/CIRCRESAHA.118.311912

17. International Diabetes Federation, IDF. Diabetes Atlas. 8 ed; 2017. Available: www.diabetesatlas.org Accessed January 29, 2019.

18. Zeng L, Ding H, Hu X, Zhang G, Gong D. Galangin inhibits α-glucosidase activity and...
formation of non-enzymatic glycation products, Food Chemistry. 2019;271:70–79. Available:https://doi.org/10.1016/j.foodchem.2018.07.148

19. da A, S. Fonseca, M.R. de A. Sartori, eds., Guia de Medicamentos, 1st ed., Martinari, São Paulo; 2017.

20. SantosCMM, FreitasM, FernandesE. A comprehensive review on xanthone derivatives as α-glucosidase inhibitors. European Journal of Medicinal Chemistry. 2018;157:1460–1479. Available:https://doi.org/10.1016/j.ejmech.2018.07.073

21. ŞöhretoğluD, SariS, BarutB, ÖzelA. Discovery of potent α-glucosidase inhibitor flavonols: Insights into mechanism of action through inhibition kinetics and docking simulations. Bioorganic Chemistry. 2018;79:257–264. Available:https://doi.org/10.1016/j.bioorg.2018.05.010

22. BagherniyaM, NobiliV, BlessoCN, SahebkarA. Medicinal plants and bioactive natural compounds in the treatment of non-alcoholic fatty liver disease: A clinical review. Pharmacological Research. 2018;130:213–240. Available:https://doi.org/10.1016/j.phrs.2017.12.020

23. PerlebergTD. Conservação ex situ e biologia reprodutiva da espinheira-santa (Maytenus ilicifolia, Celastraceae), Tese apresentada ao Programa de Pós-Graduação em Agronomia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Ciências, Universidade Federal De Pelotas; 2017.

24. Júnior, Osmar Tomazelli et al. Survival of white spot syndrome virus-infectedLitopenaeus vannamei fed with ethanol extract of Uncaria Tomentosa. Journal of the World Aquaculture Society. 2018;49(1):165–174. Available:https://doi.org/10.1111/jwas.12483

25. KoşarM, DormanHJD, HiltonenR. Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. Food Chemistry. 2005;91:525–533. Available:https://doi.org/10.1016/j.foodchem.2004.06.029

26. PanseraMR, SantosACA, PaeseK, WasumR, RossatoM, RotaLD, PaulettiG.F, SerafiniLA. Análise de taninos totais em plantas aromáticas e medicinais cultivadas no Nordeste do Rio Grande do Sul, Revista Brasileira de Farmacognosia. 2003;13:17–22. Available:https://doi.org/10.1590/S0102-695X2003000100002

27. MensorLL, MenezesFS, LeitãoGG, ReisAS, dos SantosTC, CoubeCS, LeitãoSG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method: Antioxidant activity in Brazilian plants. Phytotherapy Research. 2001;15:127–130. Available:https://doi.org/10.1002/ptr.687

28. PereiraDF, CazorrolliIH, LavadoC, MengattoV, FigueiredoMSRB, GuedesA, PizzolattiMG, SilvaFRMB. Effects of flavonoids on α-glucosidase activity: Potential targets for glucose homeostasis, Nutrition. 2011;27:1161–1167. Available:https://doi.org/10.1016/j.nut.2011.01.008

29. BoathAS, StewartD, McDougallGJ. Berry components inhibit α-glucosidase in vitro: Synergies between acarbose and polyphenols from black currant and rowanberry. Food Chemistry. 2012;135:929–936. Available:https://doi.org/10.1016/j.foodchem.2012.06.065

30. LowryOH, RosebroughNJ, FarrAL, RandallRJ. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 1951;193:265–275.

31. WangT, LiQ, BiK. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. Asian Journal of Pharmaceutical Sciences. 2018;13:12–23. Available:https://doi.org/10.1016/j.ajps.2017.08.004

32. L.M. de Souza, CiprianiTR, IacominiM, GorinPAJ, SassakiGL. HPLC/ESI-MS and NMR analysis of flavonoids and tannins in bioactive extract from leaves of Maytenus ilicifolia. Journal of Pharmaceutical and Biomedical Analysis. 2008;47:59–67. Available:https://doi.org/10.1016/j.jpba.2007.12.008

33. BaggioCH, FreitasCS, MayerB, dos SantosAC, TwardowskiA, PotrichFB, CiprianiTR, L.M. de Souza, SassakiGL, IacominiM, MarquesMCA, Mesia-VelaS. Muscarinic-dependent inhibition of gastric emptying and intestinal motility by fractions of Maytenus ilicifolia Mart ex. Reissek, Journal of Ethnopharmacology.
2009;123:385–391. Available:https://doi.org/10.1016/j.jep.2009.03.037
34. EckerA, LossCG, AdefehgaSA, BoligonAA, RomanSS. Safety evaluation of supratherapeutic dose of Maytenus ilicifolia Mart. ex Reissek extracts on fertility and neurobehavioral status of male and pregnant rats. Regulatory Toxicology and Pharmacology. 2017;90:160–169. Available:https://doi.org/10.1016/j.yrtph.2017.09.007
35. PanićM, Radić StojkovićM, KraljićK, ŠkevinD, Radojičić Redovniković, Gaurina SrčekV, Radošević. Ready-to-use green polyphenolic extracts from food by-products. Food Chemistry. 2019;283:628–636. Available:https://doi.org/10.1016/j.foodchem.2019.01.061
36. ZhangB, XingY, WenC, YuX, SunW, XiuZ, DongY. Pentacyclic triterpenes as α-glucosidase and α-amylase inhibitors: Structure-activity relationships and the synergism with acarbose. Bioorganic & Medicinal Chemistry Letters. 2017;27:5065–5070. Available:https://doi.org/10.1016/j.bmcl.2017.09.027
37. HamzaG, EmnaBH, YeddesWS, DhouafiZ, MouridAT, H. El Akrem. Chemical composition, antimicrobial and antioxidant activities data of three plants from Tunisia region: Erodium glaucophyllum, Erodium hirtum and Erodium guttatum, Data in Brief. 2018;19:2352–2355. Available:https://doi.org/10.1016/j.dib.2018.07.005
38. PessutoMB, I.C. da Costa, A.B. de Souza, Nicol initiatedF, J.C.P. de Mello, PetereliF, LuftmannH. Atividade antioxidante de extratos e taninos condensados das folhas de Maytenus ilicifolia Mart. ex Reiss., Química Nova. 2009;32:412–416. Available:https://doi.org/10.1590/S0100-40422009000200027
39. LaddhaAP, KulkarniYA. Tannins and vascular complications of Diabetes: An update. Phytomedicine. 2019;56:229–245. Available:https://doi.org/10.1016/j.phymed.2018.10.026
40. Santos-OliveiraR, Coulaud-CunhaS, ColaçoW. Revisão da Maytenus ilicifolia Mart. ex Reissek, Celastraceae. Contribuição ao estudo das propriedades farmacológicas. Revista Brasileira de Farmacognosia. 2009;19:650–659. Available:https://doi.org/10.1590/S0102-695X2009000400025
41. BahukhandiA, DhyaniP, BhattID, RawalRS. Variation in Polyphenolics and Antioxidant Activity of Traditional Apple Cultivars from West Himalaya, Uttarakhand. Horticultural Plant Journal. 2018;4:151–157. Available:https://doi.org/10.1016/j.hpj.2018.05.001
42. KoHC, LeeJY, JangMG, SongH, KimSJ. Seasonal variations in the phenolic compounds and antioxidant activity of Sasa quelpaertensis. Industrial Crops and Products. 2018;122:506–512. Available:https://doi.org/10.1016/j.indcrop.2018.06.031
43. BishnoiN, ShrivastavaB, BairwaR, Kumar SahS. Evaluation of anti-hyperglycaemic activity of Maytenus emarginatus wild leaves extract on streptozotocin-induced diabetes in wistar rats. International Journal of Pharmaceutical Sciences and Research. 2016;7:2625–2631. Available:https://doi.org/10.13040/IJPSR.0975-8232.7(6).2625-31
44. HarveyRA, FerrierDR. Bioquímica ilustrada, 5th ed., Artmed, Porto Alegre; 2012.
45. ChinsembuKC. Diabetes mellitus and nature’s pharmacy of putative anti diabetic plants. Journal of Herbal Medicine; 2018. Available:https://doi.org/10.1016/j.hermed.2018.09.001
46. YinZ, ZhangW, FengF, ZhangY, KangW. α-Glucosidase inhibitors isolated from medicinal plants. Food Science and Human Wellness. 2014;3:136–174. Available:https://doi.org/10.1016/j.fshw.2014.11.003
47. MauldinaMG, SauriasariR, ElyaB. α-Glucosidase inhibitory activity from ethyl acetate extract of Antidesma bunius (L.) Spreng Stem Bark Containing Triterpenoids. Pharmacogn Mag. 2017;13:590–594. Available:https://doi.org/10.4103/pm.pm_2017_17
48. AlongiM, VerardoG, GorassiniA, AneseM. Effect of pasteurization on in vitro α-glucosidase inhibitory activity of apple juice. LWT. 2018;98:366–371. Available:https://doi.org/10.1016/j.lwt.2018.08.065
49. AlongiM, AneseM. Effect of coffee roasting on in vitro α-glucosidase activity: Inhibition and mechanism of action. Food Research International. 2018;111:480–487.
50. Kadouh HC, Sun S, Zhu W, Zhou K. α-Glucosidase inhibiting activity and bioactive compounds of six red wine grape pomace extracts. Journal of Functional Foods. 2016;26:577–584. Available: https://doi.org/10.1016/j.jff.2016.08.022

51. Muhlack RA, Potumarthi R, Jeffery DW, Sustainable wineries through waste valorisation: A review of grape marc utilisation for value-added products, Waste Management. 2018;72:99–118. Available: https://doi.org/10.1016/j.wasman.2017.11.011

52. Šavikin K, Živković J, Alimpić A, Zdunić G, Janković T, Duletić-Laušević S, Menković N. Activity guided fractionation of pomegranate extract and its antioxidant, antidiabetic and antineurodegenerative properties. Industrial Crops and Products. 2018;113:142–149. Available: https://doi.org/10.1016/j.indcrop.2018.01.031

53. Mariot MP, Barbieri RL, Sinigaglia C, Ribeiro MV. Variabilidade em matrizes de acessos de espinheira-santa, Ciência Rural. 2008;38:351–357.

54. Iopp M. Avaliação morfoanatômica, doseamento de constituintes químicos e estudo das possíveis variações genéticas para espinheira santa (Maytenus ilicifolia mart. ex reiss.), Monografia (Conclusão do curso de Farmácia), Universidade Comunitária da Região de Chapecó; 2009.

55. Delfine S, Marrelli M, Conforti F, Formisano C, Rigano D, Menichini F, Senatore F. Variation of Malva sylvestris essential oil yield, chemical composition and biological activity in response to different environments across Southern Italy. Industrial Crops and Products. 2017;98:29–37. Available: https://doi.org/10.1016/j.indcrop.2017.01.016

56. Asgari Lajayer B, Ghorbanpour M, Nikabadi S. Heavy metals in contaminated environment: Destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. Ecotoxicology and Environmental Safety. 2017;145:377–390. Available: https://doi.org/10.1016/j.ecoenv.2017.07.035

© 2020 Schindler et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here: http://www.sdiarticle4.com/review-history/60984