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ANTIOXIDANT ACTIVITY OF HYDROALCOOLIC EXTRACTS OF GREEN AND RIPE SCARLET EGGPLANTS (*SOLANUM AETHIOPICUM* GR. GILO)

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ABSTRACT

The scarlet eggplant or 'jiló' fruit (*Solanum aethiopicum* gr. Gilo) has a characteristic pleasant bitter taste, low energy value and high content of dietary fibers. The fruits are frequently eaten green and when ripe, they are considered unsuitable for consumption and commercialization. Phytochemical studies of scarlet eggplant are rare and little is known about the polyphenols present in this fruit. The extraction procedure with an ethanol-water solution (70:30) of our samples allowed extract yields of 29% and 35% for the green and ripe fruit, respectively. The total phenolic content (TPC) of immature and ripe hydroethanolic fruit extracts were 4.46 ± 0.33 and 6.52 ± 0.01 µg GAE/mg of extract, respectively. Likewise, the antioxidant capacities evaluated by the FRAP, hydroxyl and DPPH assays were higher for the ripe fruit extract, which is likely related to the superior TPC value presented by this sample. The data set obtained in this work confirms that the green fruit of scarlet eggplant is rich in antioxidant polyphenols. Although ripe 'jiló' fruits present hardened seeds and are considered out of commercial standard, our results indicate, at least in principle, that these by-products are potential sources of polyphenols to antioxidant supplements manufacture.

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INTRODUCTION

Several benefits have been attributed to phenolic compounds present in vegetables. Epidemiological, clinical, *in vivo* and *in vitro* studies show multiple biological activities related to dietary phenolic compounds, among which antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic properties (Martins, Barros, & Ferreira, 2016; Francelin *et al*., 2018; Corrêa *et al*., 2020). Consumption of foods rich in phytochemicals are, therefore, important for the prevention of chronic diseases such as cancer, diabetes and cardio-vascular disorders, which are often associated with oxidative stress (Corrêa *et al*., 2018; Kumar & Goel, 2019; Vieira *et al*., 2020). *Solanum aethiopicum* L. gr. Gilo (syn. *Solanum gilo* Raddi) is an edible vegetable crop belonging to the family Solanaceae that was brought to Brazil by the African slaves (da Silva *et al*., 2017). Its fruit, popularly known as 'scarlet eggplant', 'gardenegg' or 'jiló', have a characteristic pleasant bitter taste and are consumed in the same way as common eggplant, i.e., as ingredients in salads, antipasti, and other savory dishes. The fruits display oval or spherical shapes, with diameters between 2.5 cm and 12 cm, and skin coloration ranging from white-green to reddish-purple, according to the plant variety or fruit ripening stage (Nwanna *et al*., 2014; San José *et al*., 2016). 'Jiló' is part of the Brazilian diet and is consumed and commercialized when physiologically immature, still having a green color (Fig. 1). However, the fruit ripens at temperatures close to 20 °C, reaching the final maturity stage in only a few days. Ripened fruits, on the other hand, present reddish color (Fig. 1), hardened seeds and greater bitterness, what in most countries makes them

unsuitable for culinary use and to be generally discarded as waste (Nwanna & Adedayo, 2017; de Alcantara *et al*., 2019). Scarlet eggplant is recommended in health diets due to its low energy value and high dietary fiber content; 100 g of the cooked fruit furnishes only 38 calories and 1.4 g of proteins. Furthermore, in some Brazilian regions, ethanolic infusions of 'jiló' are used in folk medicine to treat flu, colds and fever, to regulate the digestive system and stimulate liver metabolism (da Silva *et al*., 2017; Miamoto *et al*., 2020). Nevertheless, there is scarce scientific evidence for these beneficial effects.
Differently to eggplant (*Solanum melongena* L), largely recognized for its bioactive compounds and health promoting properties (Gürbüz *et al*., 2018), studies on the phytochemical composition and biological activities of scarlet eggplant are still rare. Taking this in consideration, our aim was to comparatively evaluate the total phenolic content and antioxidant potential of green and ripe fruits of *Solanum aethiopicum* gr. Gilo. To achieve this, samples were extracted with an ethanol-water solution (70:30) and the antioxidant capabilities of the obtained extracts were assessed using a set of three distinct *in vitro* chemical methods.

MATERIALS AND METHODS

Plant material: Green and ripe *Solanum aethiopicum* gr. Gilo fruits were obtained from a local producer of Maringá City, Paraná, Brazil. The fruits were washed under running water and dried at room temperature. Then, the material was cut into thin slices, dried at 40 ºC in an oven with forced circulation until constant weight, crushed with the aid of a blender and sieved to obtain a homogeneous fraction of 20 mesh.

Standard and reagents: Ethanol, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteau reagent, ascorbic acid, 2,4,6-tripyridyl-s-triazine (TPTZ), hydrochloric acid, ferric chloride, potassium persulfate, salicylic acid, iron sulphate, and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich Co (St Louis, MO). All other reagents used in the experiments were of analytical grade.

Extracts preparation: To 10 g of each plant material, 100 mL of an ethanol-water mixture (70:30) was added. The mixtures were kept under stirring at 100 rpm for 5 h, filtered through a Buchner funnel with the aid of a vacuum pump. Ethanol was removed using a rotatory evaporator and the extracts were lyophilized. The materials were kept in a freezer at -20 ºC until use.

Determination of total phenolic contents: Total soluble phenolic compounds in the scarlet eggplants extracts were measured according to the Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, 2.0 mL of a properly diluted sample was added to 300 µL of sodium carbonate (1.9 M Na_2CO_3) and 100 µL of Folin reagent (1 M). The mixture was left to stand for 1 hour in the dark and the absorbance determined at 725 nm in a spectrophotometer (Shimadzu). Gallic acid was used as a standard to construct a calibration curve. Analyses were performed in triplicates and results were expressed in µg of GAE per mg extract.

Antioxidant activity evaluation via chemical methods: Three different *in-vitro* methods were used to estimate the antioxidant potential of extracts: (1) reduction power of the ferric ion (FRAP); (2) reduction of the 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH_{\bullet}$), and (3) hydroxyl radical scavenging activity. The FRAP assay was carried out following the protocol of Koehnlein *et al*. (2016). To prepare the FRAP solution, 25 mL of acetate buffer (300 mmol/L) were mixed with 2.5 mL of a TPTZ solution (2,4,6-Tris 2-pyridyl-s-triazine) (10 mmol/L) and 2.5 mL of the ferric chloride solution (20 mmol/L). To the 900 µL of the FRAP reagent in an assay tube, 90 µL of distilled water were added plus 30 µL of sample at different concentrations. After 30 min of reaction at 37 °C in the dark, reading was done at 595 nm. Standard curves were constructed with Trolox ($R^2 = 0.99$) and the results were expressed as mmol trolox equivalents (TE)/mg lyophilisate material. The free radical scavenging activity (DPPH) was evaluated as previously described by Soares *et al*. (2009) and Corrêa *et al*. (2017). Firstly, a stock solution of DPPH was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and stored at -20 °C until use. After, the DPPH working solution was prepared: 10 mL of the DPPH stock solution was added to 45 mL of methanol. Next, 150 µL of the sample and 2850 µL of the DPPH working solution were added to a tube.

Reaction took place for 1 hour at room temperature in the absence of light. As a negative control of the reaction, distilled water was used instead of the sample. Then, absorbance was determined at 515 nm. A 0.02% butyl hydroxy toluene (BHT) solution was used as a positive control. To calculate the percentage of DPPH discoloration, the following equation was used: $[(Abs_{control} - Abs_{sample)}/Abs_{control}] \times 100$. The lyophilisate concentrations (mg/mL) providing 50% antioxidant activity were calculated from the graphs of antioxidant activity against the sample concentrations. Trolox was used as a positive control and water was used as negative control. The results were expressed as IC_{50} values (sample concentration providing 50% of antioxidant activity). The hydroxyl radical scavenging activity of extracts was measured having as principle the Fenton's reaction, according to the work of Mu *et al*. (2012). For the assay, 0.5 mL of salicylic acid-ethanol solution (9.1 mM), 0.5 mL of sample solution at different concentrations, 0.5 mL of $FeSO_4$ solution (9.1 mM) and 3.0 mL of distilled water were consecutively mixed in a tube. To start the reaction, 3.0 mL H_2O_2 (8.8 mM) was added to the tube, and the absorbance at 510 nm was read. The hydroxyl radical scavenging activity percentage was calculated using the formula: $[(Abs_{sample} - Abs_{control})/Abs_{blank}] \times 100$. The results were also expressed as IC_{50} values. Ascorbic acid was used as positive control whereas the negative control was water.

Statistical analysis: Three repetitions of the sample and triplicates for each concentration were carried out in all assays. The results were reported as mean ± standard error. The IC_{50} values and graphics were obtained from the logarithmic non-linear regression curve derived from the plotted data using the GraphPad Prism software (version 8.0).

RESULTS AND DISCUSSION

The extraction of phenolic molecules from vegetable matrices is highly dependent on the polarity of the solvent, as a polar component is easily extracted using a polar solvent, which will inclusively influence the quantity and quality of the final extract (Maimoto *et al*., 2019). In this sense, Dent *et al*. (2013) claim that hydroethanolic solutions are suitable media for the

Table 1. Total phenolic content and antioxidant capacities of hydroalcoholic extracts from green and ripe scarlet eggplants

	Green fruit	Ripe fruit
Total phenolic content (mg GAE/g extract)	4.46±0.33[(a)]	6.52±0.01[(b)]
Antioxidant activity		
FRAP (µM TE/mg extract)	71.89±0.70[(a)]	94.19±1.64[(b)]
Hydroxil assay (IC_{50} µg/mL)	1224.45±13.26[(a)]	753.96±8.00[(b)]
DPPH assay (IC_{50} µg/mL)	425.06±18.08[(a)]	333.71±4.21[(b)]

Means with different letters on the same line differ significantly ($p<0.05$).

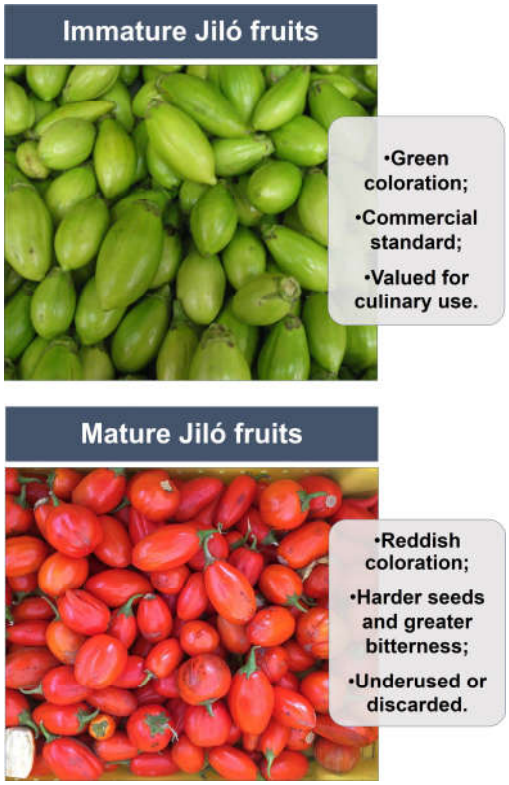

Figure 1. Immature and ripened scarlet eggplant (jiló) fruits (*Solanum aethiopicum* gr. Gilo).

extraction of phenolic compounds owing to the distinct polarities of the bioactive molecules and their acceptability as a food grade solvent. The extraction procedure with an ethanol-water solution (70:30) of our samples allowed extract yields of 29% and 35% for the green and ripe fruit, respectively. Recently, Miamoto *et al*. (2020) optimized the extraction of polyphenols from scarlet eggplant using response surface methodology. They verified that the ethanolic degree was the most important parameter in the extraction procedure, and achieved the best results of phenolic recovery when using a 61% hydroalcoholic solution.

It has been established that phenolic components are the major phytochemicals with antioxidant activity, and this activity is due to their redox actions (Garcia *et al*., 2020). Wherefore, the total phenolic content (TPC) of the hydroethanolic 'jiló' extracts were assessed and the results are shown in Table 1. The TPC value found for the ripe fruits was 1.46-fold the value found for the immature fruits. Nwanna *et al*. (2014), in their study on *S. aethiopium* species from Nigeria, reported a less expressive TPC value (2.83 ± 0.19 mg GAE/g) for an aqueous extract of 'jiló'. However, Miamoto *et al*. (2020) found TPC values between 1.75 ± 0.07 and 7.21 ± 0.05 mg GAE/g of dry scarlet eggplant, therefore, within the range observed in the present work. The use of more than two techniques for evaluating the antioxidant capacity of vegetable extracts is mandatory, as antioxidant molecules act by distinctive mechanisms, each possessing its specific target within the reaction matrix (Corrêa *et al*, 2014; Gonçalves *et al*., 2019). Such heterogeneous chemical reactivity implies in varying degrees of antioxidant action in the chemical methods (Correa *et al*., 2017). To our best knowledge, this is the first study comparing the antioxidant potentials of *S. aethiopicum* gr. Gilo fruits of different ripening stages. Moreover, such set of antioxidant techniques was never used to evaluate scarlet eggplant samples. The antioxidant capacities evaluated by the FRAP, hydroxyl and DPPH assays were higher for the ripe fruit extract (Table 1). This greater antioxidant potential is likely related to the superior TPC value presented by this sample. Our DPPH radical scavenging activities were similar to those found by Nwanna & Adedayo (2017) for dried samples of ripe and unripe 'jiló', with IC_{50} values of, respectively, 420 and 375 µg/mL. Likewise, Miamoto *et al*. (2020) found IC_{50} values between 270 and 440 µg/mL when assessing immature 'jiló' extracts obtained via sonication with distinct ethanolic degrees and extraction times.

Conclusion

The data set obtained in this work confirms that the green fruit of *S. aethiopicum* gr. Gilo is rich in antioxidant polyphenols. Although ripe 'jiló' fruits present hardened seeds and are considered out of commercial standard, our results indicate, at least in principle, that these by-products are potential sources

of polyphenols to antioxidant supplements manufacture. Furthermore, due to its bitter taste, the scarlet eggplant is not appreciated by children and even adults, and the development of a nutraceutical supplement would allow this public to benefit from its bioactive compounds. However, considering that the ripe fruits usually present greater bitterness than the green fruits, encapsulation approaches may be necessary to minimize this undesired characteristic.

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