ABSTRACT

Passiflora edulis Sims var. flavicarpa O. Deg. (Yellow-passion fruit) is the native plant species most used by juice industries in Brazil while its leaf extracts are widely employed in folk medicine. This study evaluated the phenolic content of leaves, roots, fruit shells and pulp of plants of P. edulis in juvenile, flowering and fruiting stages. The extent of scavenging and/or degradation of reactive nitrogen and oxygen species by plant extracts was also investigated. Leaves were the organs that most accumulated phenolics/flavonoids, regardless of plant developmental stage. Leaf extracts efficiently scavenged DPPH by up to 67 % while root and fruit shell extracts effectively captured up to 80 % O$_2$. Maximum activity of catalase (51.6 mmol H$_2$O$_2$ min$^{-1}$ mg prot$^{-1}$) and ascorbate peroxidase (2.2 mmol ascorbate min$^{-1}$ mg prot$^{-1}$) was recorded in leaf extracts from plants in the fruiting stage. Superoxide dismutase activity reached its highest levels (37.5 U min$^{-1}$ mg prot$^{-1}$, on average) in plant leaves of both juvenile and fruiting plants. Overall, these results suggest that, for therapeutic purposes, parts of P. edulis should be harvested when plants are in the fruiting stage due to the excellent antioxidant properties of their extracts and their accumulation of phenolic compounds.

Keywords: antioxidant enzymes, DPPH, flavonoids, passion fruit, secondary metabolism, superoxide anion

Introduction

The genus Passiflora (family Passifloraceae) includes about 600 plant species (Ayres et al. 2015; Wosch et al. 2017) distributed in the tropical and subtropical regions around the world (Ayres et al. 2015). Over 140 species were described to occur in Brazil, in which 83 of them are considered endemic (Gomes et al. 2017) and 60 species produce edible fruits (Pertuzatti et al. 2015). Passiflora edulis var. flavicarpa (yellow passion fruit) is a perennial vine that bears trilobate, toothed-edged leaves. A lonely flower emerges from each node surrounded by three green bracts containing five sepals and white petals, purple corolla in the base and five stamens with large anthers (Zibadi & Watson 2004). Passiflora edulis is the native species (Zibadi & Watson 2004) most cultivated and used in Brazil by juice industries (Pertuzatti et al. 2015). Cultivation of P. edulis in Brazil for commercial purposes started in the early 1980s and was expanded to date by family farming (Meletti 2011).

Extracts of Passiflora species leaves have been used in folk medicine for treating neurosystem disorders, such as anxiety, migraine and insomnia (Zibadi & Watson 2004; Ayres et al. 2015). Ethnobotanical studies show that the fruit pulp is used as cardiac tonic, moderate diuretic and digestive stimulant and to treat asthma, bronchitis, whooping cough and urinary infections (Zibadi & Watson 2004). Indeed, P. edulis was included in 2009 by the Unified Health System (SUS; Brazil) in the Brazilian National List of Medicinal
Plants of Interest (MS 2009). A year later, P. edulis was also included in the Brazilian Pharmacopoeia (Brasil 2010).

Several secondary metabolites were reported to occur in leaves of P. edulis, but the phenolic contents in root, fruit shell and pulp remain to be evaluated. Besides this, the potential as antioxidant of extracts from different parts of such species will comprise valuable information to rationalize the folk use of P. edulis to treat several diseases. This work evaluated the accumulation of phenolic and polyphenolic (flavonoid) compounds in different parts of P. edulis plants in distinct development stages. The potential of ethanolic extracts of parts of P. edulis to scavenge free radicals were also investigated, together with the endogenous activity of antioxidant enzymes in plant leaves.

Materials and methods

Plant material and experimental design

Samples were harvested from plants cultivated in Pitangueiras Farm located in Sooretama, Espírito Santo, Brazil (19°12'05.6''S 40°03'38.5''W), unless otherwise stated. Plant parts were harvested during the phenological stages juvenile (Dec 2017; leaves and roots), flowering I (Feb 2017; leaves and roots), flowering II (Sep 2017; leaves and roots), fruiting I (Nov 2016; leaves, roots and fruits) and fruiting II (Dec 2017; leaves, roots and fruits) (Fig. 1). Shell and pulp were also separated from the fruits for subsequent analyses. Plants in juvenile stage were characterized by those grown in a greenhouse at Incaper (19°25’01.2’’S 40°04’44.1’’W) at Tmin of 24.1 °C and Tmax of 29.2 °C, for 65 days before the first flowering event. The species was identified and the voucher specimen deposited in the herbarium of the Department of Botany at the Federal University of Minas Gerais under the number BHCB 184739.

The experimental design was completely randomized, in which the indicated plant parts were harvested from eight individuals (each defined as a biological sample) in the following developmental stages: juvenile, flowering I and II and fruiting I and II. Pluviometric and temperature variations were monitored on a monthly basis by Incaper Weather Station (Linhares, Espírito Santo, Brazil) during the period by which Passiflora edulis Sims var. flavicarpa O. Deg plants were investigated (Fig. 2). Completely mature fruits were harvested immediately after natural detachment from the plants (Faleiro et al. 2005).

Ethanolic extracts preparation

The extracts were prepared by adding to intact tissues a volume of ethanol PA (in mL) correspondent to 10 times the organ weight in grams. After 72 h in sealed flasks in the absence of light Samples (Brasil 2010; Shah et al. 2004; Shelar et al. 2018; Carvalho et al. 2019), samples were filtered and the organic fraction dried at < 50 °C (Dai & Mumper 2010; Shelar et al. 2018) to obtain the corresponding solid
residues. The extracts yield was determined considering the plant material fresh weight and the mass of the dried organic fraction. One milligram of each dry extract was resuspended in 1 mL of ethanol absolute and the ethanolic extract used in the subsequent analysis.

Quantification of total phenolic compounds and total flavonoids in ethanolic extracts

The determination of phenolic compounds was performed as previously described (Murthy et al. 2002). Briefly, 1-volume of sample was mixed to 5-volume of 1X Folin Ciocalteu reagent and 4-volume of 7.5 % sodium carbonate. Each system was incubated for 30 min at room temperature and 150 rpm following analysis at 765 nm. Tannic acid (0 – 800 µg mL⁻¹) was used as a standard (Box 1983; Blainski et al. 2013) and the total phenolics compounds expressed as equivalents of tannic acid/dry extract.

The total flavonoid content was determined in the same samples following standard procedures (Jayaprakasha et al. 2001). One volume of ethanolic extract was added to 2.5-volume of 4 % HCl and 2.5-volume of 10 % vanillin. Each system was incubated for 30 min at room temperature and 150 rpm and analyzed at 460 nm. Quercetin (0 – 800 µg mL⁻¹) was used as a flavonoid standard and the results were expressed as equivalents of quercetin/dry extract.

Scavenging of reactive nitrogen species

The potential of ethanolic extracts of *P. edulis* (1 mg mL⁻¹) to scavenge reactive species of oxygen was investigated against anion superoxide radicals (Silva et al. 2012). Superoxide anions were artificially generated in a system containing 1 µM EDTA, 17 µM L-methionine, 10 µM NBT and 2 µM riboflavin in the presence or absence of plant extract (1:1) after incidence of fluorescent light for 10 min at 25°C. Control reactions were incubated for 10 min, at 25 °C in the dark. The absorbance was monitored at 525 nm and resveratrol was used as positive control.

Activity of antioxidant enzymes in leaves of *P. edulis*

The activity of ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) in leaves of *P. edulis* was assessed essentially as previously reported (Silva et al. 2017). The molar extinction coefficients (ε) of 2.8 mM⁻¹ cm⁻¹ and 39.4 mM⁻¹ cm⁻¹ were used to determine the amount of oxidized ascorbate and degradate H2O2 for the estimation of APX and CAT activities, respectively. One unit of SOD refers to the amount of SOD necessary to inhibit the reduction of nitroblue tetrazolium (NBT) by 50 %. The total protein content in leaf samples was determined according to the method of Bradford (Bradford 1976).

Statistical analyses

Data were submitted to Shapiro-Wilk to check the normality and F test to verify the distribution using the ASSISTAT software (Silva & Azevedo 2016). Data from organs of plants in each developmental stage were individually submitted to analysis of variance and mean test (Scott-Knott; *P* < 0.01) using ASSISTAT software (Silva & Azevedo 2016).

Results

Total phenolics/flavonoids in *P. edulis* extracts

Leaves of *P. edulis* plants in juvenile (122.0 µg tannic acid mg⁻¹ dry extract), fruiting I (710.0 µg tannic acid mg⁻¹ dry extract) and flowering I (571.0 µg tannic acid mg-1 dry extract) were harvested from June 2016 to Dec 2017 at Pitangueiras Farm (Sooretama, Brazil), interval by which plant samples were harvested as indicated. Juvenile plants were grown at greenhouse during the same period that field plants were in fruiting stage II. Data were gathered by Incaper Weather Station locted in Linhares (Espírito Santo, Brazil).
Figure 3. Total phenolic (black bars) and flavonoid (gray bars) contents in Passiflora edulis in various developmental stages. Ethanolic extracts (1 mg mL⁻¹) were prepared from plant samples harvested in juvenile (A), fruiting I (B), flowering I (C), flowering II (D) and fruiting II (E) stages. Values correspond to the means (n = 8) + standard deviations. Total phenolics are expressed as tannic acid equivalents while total flavonoids as presented as quercetin equivalents. Distinct uppercase letters indicate significant differences in the phenolic contents while distinct lowercase letters indicate significant differences in flavonoid contents (Scott-Knott; P < 0.01) within a phenological stage. DE, dry extract.
extract) stages presented the highest levels of phenolic compounds among the studied plant organs, regardless of the plants development stage (Fig. 3). Leaves from *P. edulis* plants presented the highest levels of flavonoids compounds when compared to the other organs (Fig. 3).

The cumulative precipitation and temperature during the harvesting of plants in flowering I stage (Nov 2016) averaged 215.8 mm and 24.3 °C, respectively (Fig. 2). Plants in flowering I stage (Feb 2017) experienced pluviometric and temperature conditions of 68.2 mm and 26.5 °C. The cumulative precipitation and temperature in Sep, 2017, when plants in flowering II stage were harvested, were on average 18.8 mm and 23.5 °C (Fig. 2). The cumulative precipitation and temperature in Dec 2017 (period of harvesting of plants in juvenile and fruiting II) were 291.4 mm and 25.8 °C, respectively (Fig. 2).

**Scavenging of reactive nitrogen and oxygen species by *P. edulis* extracts**

Ethanolic extracts from leaves (1 mg mL⁻¹) in juvenile, flowering I and II and fruiting I and II stages effectively scavenged 53.7, 66.3, 50.8, 64.8 and 50.2 % of the reactive nitrogen species DPPH present in the reaction medium, respectively (Fig. 4). In contrast, root extracts were notable scavengers of the reactive oxygen species O₂⁻ as they capture 73.1 % (juvenile plants), 61.3 % (flowering I plants), 78.1 % (flowering II plants), 75.0 % (fruited I plants) and 59.6 % (fruited II plants) of the free radicals formed in the reaction medium (Fig. 5). The potential of extracts from fruit shells (fruited I stage) to scavenge O₂⁻ was comparable to that of root extracts from the same plants while root and leaf extracts from plants in flowering II stage were equally effective against O₂⁻ (Fig. 5).

Among the plant parts and developmental stages investigated, leaf extracts from plants in flowering I and fruiting I stages exhibited the highest capacity to scavenge reactive nitrogen species (Tab. 1); Extracts from fruit pulp (fruited II) were as efficient as extracts from roots (juvenile stage), fruit shells and roots (fruited I) and leaves and roots (flowering II) with respect to the scavenging of reactive oxygen species (Tab. 1).

**Activity of antioxidant enzymes in leaves of *P. edulis***

The activity of SOD was 2.3-fold higher in leaves of *P. edulis* in flowering I stages (32.3 U min⁻¹ mg prot⁻¹ on average) than that of leaves from flowering II stages (13.9 U min⁻¹ mg prot⁻¹ in average) (Fig. 6). The APX and CAT activities showed similar profiles among the plant parts. The highest CAT and APX activities were observed in leaves of fruiting I plants (51.6 mmol H₂O₂ min⁻¹ mg prot⁻¹ and 2.2 mmol ascorbate min⁻¹ mg prot⁻¹) followed by juvenile plants (36.5 mmol H₂O₂ min⁻¹ mg prot⁻¹ and 1.4 mmol ascorbate min⁻¹ mg prot⁻¹) and the remainder stages (21.7 mmol H₂O₂ min⁻¹ mg prot⁻¹ and 0.9 mmol ascorbate min⁻¹ mg prot⁻¹, on average).

**Discussion**

The level of secondary metabolites is reported to vary among the organs of a plant species and according to the environmental conditions and plant development stage (Deborde et al. 2017). The low content of phenolic compounds in plants in the juvenile stage may be attributed to the lower tissue lignification and therefore lower amounts of lignin precursors in cell wall when compared to mature plants (Ncube & Staden 2015). Likewise, lower levels of phenolic compounds were found in leaves of *Vaccinium macrocarpon* (cranberry) cv. ‘Stevens’ Ait. during budding stage than that of cranberry plants in flowering and fruiting stages (Berezina et al. 2017). The levels of phenolic compounds diminished in *P. edulis* leaves and roots as plants began to flower until fruiting stage, a phenomenon that was also observed in cranberry leaves during flowering (Berezina et al. 2017). In fact, flavonoids can account for up to 4% of pollen dry weight, provide color to pollen and petals and contribute to the attraction of pollinators (Theis & Lerdau 2003; Ferreyra et al. 2012). Also, the structure of *P. edulis* flowers is quite complex, whose crown’s diameter can exceed 12 cm and present several pollen grains and aromas (Jesus et al. 2017). Ecological studies have shown a positive correlation with respect to secondary metabolites among the different plant parts, in which increased amounts are found in the phloem, followed by leaves and nectar (Parachnowitsch et al. 2017).
Figure 4. Scavenging of DPPH radicals by ethanolic extracts from *Passiflora edulis* plants in various developmental stages. Ethanolic extracts (1 mg mL⁻¹) were prepared from samples harvested in juvenile (A), fruiting I (B), flowering I (C), flowering II (D) and fruiting II (E) stages. Values correspond to the means (n = 8) + standard deviations. Distinct letters indicate significant differences among the plant parts (Scott-Knott; *P* < 0.01).
Figure 5. Scavenging reactive oxygen species by ethanolic extracts from *Passiflora edulis* plants in various developmental stages. Ethanolic extracts (1 mg mL\(^{-1}\)) were prepared from plant samples harvested in juvenile (JUV), flowering I and II (FLO I and FLO II) and fruiting (FRU I and FRU II) stages. Values correspond to the means (n = 8) + standard deviations. Distinct letters indicate significant differences (Scott-Knott; P < 0.01).
Phenolic content and antioxidant activity of parts of *Passiflora edulis* as a function of plant developmental stage

In contrast, the lower levels of phenolics in leaves and roots of plants in fruiting II stage in comparison with those of plants in flowering II stage may result from allocation of these secondary metabolites to fruits. Also, plants in flowering II and fruiting II stages were senescent, which may explain the highest levels of total phenolic compound/flavonoid in fruit shells and pulp. Atypical climate conditions, characterized by a 3.5-fold increase in rainfall in August/September 2017 in relation to the same period in 2016, were registered prior to the harvesting of plants in flowering II stage. Such condition could lead to plant stress. The increase in the flavonoid contents in organs of senescent *P. edulis* is likely a response to oxidative stress generated during plants aging (Ferreira et al. 2012).

The differences in the performance of ethanolic extracts of leaf and root to scavenge DPPH and O$_2^-$ radicals can be explained by the fact that a given secondary metabolite may be chemoselective for reactive nitrogen species in detriment of reactive oxygen species. This has been demonstrated for resveratrol, a grape-accumulating polyphenol that is chemoselective to DPPH and, therefore, a poor O$_2^-$ scavenger (Silva et al. 2012; Liberto et al. 2017; Das et al. 2018). The efficiency of *P. edulis* extracts to scavenge DPPH or O$_2^-$ can be partly explained by the high phenolic content in the samples. Secondary metabolites other than flavonoids appear to contribute to the ability of *P. edulis* extracts to capture free radicals since extracts containing the highest levels of flavonoids were not necessarily the most efficient to scavenge DPPH or O$_2^-$. Expressive O$_2^-$ scavenging activity was observed for root and fruit shell extracts. Fruit shells, together with *P. edulis* seeds, account for up to 70% of fresh fruit weight and they are disposed of by industries during the production of passion fruit juice (Oliveira et al. 2002). In this sense, the fruit shell, a juice industry by-product, could be considered for further use as free radical scavenger.

**Figure 6.** Activity of antioxidant enzymes in leaves of *Passiflora edulis* plants in various developmental stages. Leaves were harvested from plants in juvenile (JUV), flowering I and II (FLO I and FLO II) and fruiting (FRU I and FRU II) stages for the evaluation of ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) activities. Values are the means (n = 8) ± standard deviations. Distinct letters indicate significant differences (Scott-Knott; P < 0.01).
The increment in CAT, APX and SOD activities in leaves of *P. edulis* in fruiting I stage indicated that plants were coping well with adverse environmental conditions since the individuals from the orchard were visibly healthy. The SOD catalyzes the dismutation of O$_2$ to H$_2$O$_2$, whose excess is converted to H$_2$O by the action of CAT and APX, in which this latter is assisted by ascorbate. Hence, *P. edulis* leaf extracts can effectively control the levels of reactive species via the activity of antioxidant enzymes and non-enzymatic oxidants such as phenolic compounds, which validates the use of *P. edulis* leaves for the treatment of oxidative-stress-driven diseases.

**Conclusion**

Leaves of *P. edulis* presented the highest phenolic compounds/flavonoids contents regardless of the plant developmental stage. The maximum accumulation of phenolics/flavonoids in roots, leaves, fruit shells and pulp occurred when plants were in the reproductive stage. Economical value is now given to fruit shells of *P. edulis*, a juice industries's waste, as phenolic compounds, which validates the use of *P. edulis* leaves if the harvesting is carried out during the fruiting stage.

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**References**


Berezina EV, Brillkina AA, Veselov AP. 2017. Content of phenolic compounds, ascorbic acid, and photosynthetic pigments in *Vaccinium macrocarpon* Ait. dependent on seasonal plant development stages and age (the example of introduction in Russia). Scientia Horticulturae 216: 139-146.

Blainski A, Lopes GC, Mello JCP. 2013. Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. Molecules 18: 6852-6865.


Brazil. 2010. Farmacopeia brasileira Vol. 2. Brasilia, ANVISA.
