Comparation of Free Radical Scavenging Activity between Seven Species of *Passiflora* L.

Montero, D.A.V

Bonfim, F.P.G
Ming, L.C

Laboratory of Medicinal Plants and Ethnobotany
Department of Horticulture
Faculty of Agronomic Science
State University of Sao Paulo (Unesp)
Botucatu
Brazil

Lima, G.P.P
Laboratory of Chemistry and Biochemistry
Department of Chemistry
Institute of Biosciences
State University of Sao Paulo (Unesp)
Botucatu
Brazil

Meletti, L.M.M.
Horticultural Research Center
Agronomic Institute
Campinas (IAC)
Brazil

Abstract

Antioxidant capacities of seven species of Passiflora were evaluated through comparation of the free radical DPPH scavenging activity. The studied species included cultivated and traditionally used P. edulis, P. incarnata and P. alata and less common species P. coccinea, P. laurifolia, P. mucronata and P. gardneri. The experimental design was completely randomized with ANOVA and Tukey test as main statistical analyses. The results showed that species of Passiflora had variable antioxidant capacities, ranging from 28 to 95% of free radical DPPH scavenging activity.

Keywords: P. alata, P. coccínea, P. gardneri, P. incarnata, P. edulis, P. laurifólia, P. mucronata, Antioxidant

1. Introduction

Natural products such as fruits, wines and teas with antioxidants properties play an important role in health through their modulating effect on the oxidative processes that occur in the body.

The formation of reactive oxygen and subsequent oxidation of biological molecules constitutes a mechanism of tissue damage present in various pathological processes such as inflammation, strokes, myocardial infarction, atherosclerosis, several degenerative diseases like Alzheimer, Parkinson and some types of cancer (OZBEN, 1998). The assay of the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) is among the simples and widely used methods to determine antioxidant potential by the free radical scavenging activity of natural products (CAO et al., 1997).

Passionflowers are mostly herbaceous or woody vines with tendrils, rarely erect herbs, small trees, or shrubs. The leaves are alternate often with nectarines on the petiole; the lamina could be simple or compound, lobed or unlobed, with venation pinnate, palmate, or rarely pedate; The flowers are axillary, solitary, rarely in a cymose inflorescence; bracts are minute to large; sepals 5; petals 5, sometimes 0 (subgenus *Decaloba*); corona with 1 to several series of filaments; operculum membranous; stamens 5 to 8, inserted on androgynophore below the ovary; the fruits are indehiscent or rarely dehiscent, often consume by birds and mammals that disperse the seeds (FEUILLET and MACDOUGAL, 2003). The vast majority of *Passiflora* species are distributed in tropical and subtropical regions of the New World with Colombia and Brazil as the main centres of diversity. Cultivated species have been introduced in many countries for fruit production and now they are naturalized beyond their native ranges.

Some species of the genus *Passiflora* L., particularly *P. edulis* Sim (both, the yellow and the purple cultivars), *P. incarnata* L. and *P. alata* Curtis are well recognize as human food and phytomedicine. Today these plants are an integral part of phytopharmaceutical products all around the world and the preparation such as tea like products are wildly commercialized and use in folk medicine from the time of Amerindian civilization (VASIC et al., 2012). Several studies on commercial *Passiflora* species have showed high antioxidant activities among other interesting properties such as antibacterial, anti-inflammatory, anti-tumor, anti-anxiety, anti-hypertensive, cytotoxic and hemolytic (DHAWAN et al., 2004; INGALE and HIVRALE, 2010; PATEL et al., 2011). Less common species, *P. nitida* and *P. palmeri* were characterized by a high antioxidant power that correlates with high catechin and *o*-diphenol contents (INGALE and HIVRALE, 2010). *P. tenuifila* show very high amounts of flavones and total phenols, but intermediate levels of antioxidant activity, probably due to the lower contribution of *o*-diphenols and gallocatechins relative to the phenol content (BENDINI et al., 2006). *P. coriacea* also demonstrated antioxidant power and *P. foetida* leaf extracts have showed a low antioxidant power and low amounts of *o*-diphenol and catechin (INGALE and HIVRALE, 2010). Others author that presented similar results and conclusions with *Passiflora* species included Bois (1958), Carvajal et al. (2011), Sasikala et al. (2011), Macoris et al. (2012) and Mekar et al. (2013).

The purpose of this study was to determine and compared the antioxidant activities of seven species of *Passiflora* including wild and cultivated plants by the 1,1-diphenyl-2-picrilhydrazyl (DPPH) method. The principle of this assay was grounded on the colorimetric property of DPPH which present a strong absorption at the visible spectrum (515 nm), characterized by an intense purple coloration. When the DPPH is in the presence of substances which are able to scavenge free radicals the absorption is inhibited and the colorations decreases. The degree of discoloration is directly correlated with the free radical scavenging activity of the evaluated samples (MATHIESEN, 1997; LEHUÉDÉ, 1999).

2. Methodology

The plant materials were collected from live plants as follows: *P. edulis* L. and *P. alata* Curtis., were collected from the orchard of the Faculty of Agronomic Science (FCA), of the State University of Sao Paulo (Unesp)-Botucatu; *P. coccinea* Aubl., *P. gardneri* Mast., *P. laurifólia* L. e *P. mucronata* Lam., were donated by the Agronomic Institute, Campinas (IAC), Brazil; and *P. incarnata* L. was donated by private producer. All materials were air dried at 47°C for 7 days. Dry leaves were separated from the stems, powdered, sifted and packed. The DPPH assay was perform in triplicates with at the Laboratory of Biochemistry, Institute of Biosciences, Unesp-Botucatu, Brazil, as follows: 5mg of each of the studied species were extracted in 10ml of ethanol, shacked and submitted to ultrasonic bath for 15 min and centrifugation for 10 min at 2000 rpm. Immediately, 500 µl of supernatant were added to 300 µl of DPPH solution [0.2mg/ml], homogenized and leave in the dark. After 35 min, the samples were read in spectrophotometer at 517 mm and compare with 3.5ml of ethanol mixed with 300 µl of the same solution of DPPH.

The experimental design was completely randomized and statistical analyses between samples were performed by ANOVA and Tukey test with the aid of the MiniTab statistical software v.14.0.

3. Results and Discussion

Significant differences between the scavenger activities of *Passiflora* were found among the studied species ($p<0.05$). All the samples presented antioxidant activity with particularly high values in *P. laurifolia* and *P. coccinea* which presented more that 95% of scavenging activity (Table 1).

Commercial species, *P. edulis* and *P. incarnata* had middle values with 50-60% of scavenging activity while *P. alata*, *P. gardneri* and *P. mucronata* showed less than 40% of scavenging activity of the free radical DPPH (Figure 1). No other works were found in our revision among scientific literature addressing the antioxidant activity of *P. gardneri* and *P. mucronata*. The decrease in absorbance of the DPPH radical caused by the antioxidant substances was due to the scavenging of the radical by hydrogen donation by antioxidant substances present in the experimental samples. The scavenging activities of the samples were visually noticeable as the color change from purple to greenish-yellow.

The antioxidant capacity of passionflowers is belied to be mainly dependent on phenolic compounds, which are an important group of secondary metabolites, synthesized in response to unfavorable condition (PITCHERSKY and GANG, 2000). As several authors have mentioned, leaf extracts of *Passiflora* are rich in polyphenols, such as vitexin, isovitexin, orientin and isoorientin (ULUBELEN et al., 1982; PETRY et al., 2001; PEREIRA et al., 2004). The antioxidant activity of phenolic compounds depends on their molecular structure, based on the availability of phenolic hydrogens, which result in the formation of phenoxyl radicals due to hydrogen donation (RAMARATHNAM et al. cited by MEKAR et al., 2013). Following Rudniki et al. (2007), there is a direct linear relationship between the total phenolic content and total antioxidant activity of *Passiflora*, indicating that the phenolic compounds might be major contributors to antioxidant activities in *Passiflora* leaf extracts. The same authors concluded that *P. alata* and *P. edulis* possess *in vitro* and *ex vivo* antioxidant activity against oxidative protein damage and should be considered as new sources of natural antioxidants. Interestingly our work reveled that *P. laurifolia* and *P. coccinea* have more antioxidant activity when compared with commercial species *P. edulis*, *P. incartata* and *P. alata* by 34% to 57% free radical DPPH scavenging activity, respectively.

4. Conclusion

The results showed that species of *Passiflora* had a variable antioxidant capacity, ranging from 28 to 95% of DPPH scavenging activity. *P. laurifolia* and *P. coccinea* have greater free radical scavenging activity than commercially used species (*P. edulis, P. incarnata* and *P. alata*) and should be considered good sources of natural antioxidants. Further studies should be address to the identification of which phenolic compounds are responsible for the antioxidant activity of *P. laurifolia* and *P. coccinea*; also, assess the way in which the substances contribute to this activity.

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Table 1. Comparation of Free Radical Scavenging Activity between Seven Species of *Passiflora.*

Species	*P. incarnata*	*P. laurifolia*	*P. gardneri*	*P. mucronata*	*P. edulis*	*P. alata*	*P. coccinea*
Absorbance	0.186 ± 0.048b	0.016 ±0.012a	0.257 ±0.073c	0.271 ±0.011c	0.146 ±0.039b	0.233 0.039±bc	0.019 ±0.003a
% DPPH scavenger activity	50.621	95.833	31.738	28.014	61.259	38.032	95.035
IC50 (g) *	0.049	0.026	0.078	0.089	0.041	0.065	0.026
TEAC (μm/g amostra)	1.242	2.340	0.783	0.693	1.500	0.936	2.321

Presented data is the mean value of the triplicate for each species. The horizontal values followed by the same latter are not significant differences according with the Tukey´s tests at level of 5% probability.
* Minor values represent the strongest antioxidant capacity

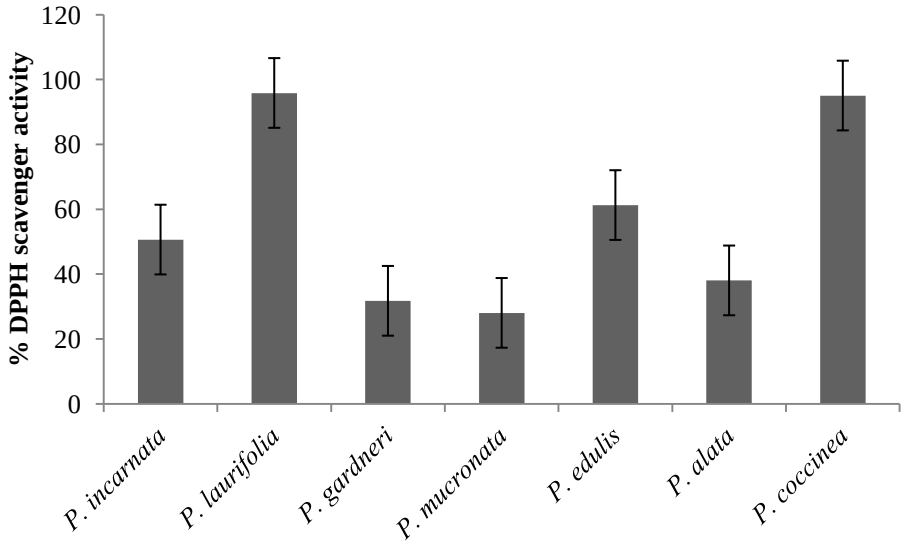

Figure 1: Comparation of Free Radical Scavenger Activity of the Evaluated Species of *Passiflora*