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In vitro Antioxidant Activity between Bioactive Compounds from Nine Species of Passiflora

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Research Article

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ABSTRACT

Main bioactive substances identified from the genus *Passiflora* include polyphenols, flavonoids, carotenoids, anthocyanins and other natural antioxidants that are critical factors for maintaining optimum health. Polyphenols mainly *C*-glycosides are present in well studied species such as *P. edulis*, *P. incarnata* and *P. alata.* However, most *Passiflora* species remains little explored and it's for this reason that we address our work at the comparison between species. Three experiments with completely randomized designs were performed in order to compare the total amount of flavonoids, phenols and *in vitro* free radical DPPH scavenger activity (%DPPH) of nine species of *Passiflora* (*P. edulis*, *P. alata*, *P. incarnata*, *P. ligularis*, *P. tripartite*, *P. coccinea*, *P. gardneri*, *P. laurifólia* and *P. mucronata*). Non parametric Kruskal-Wallis and Friedman tests (for flavonoids), parametric analyses of variances (Anova) and the last significant differences (LSD) tests (for phenols and free radical scavenger activity) were performed to compare the studied species and then a correlation analysis was carried out to assess the interaction between the variables using RStudio statistical software. Results showed significant differences between the studied passionflowers for the total amount of flavonoids (p=4.4e-5), total amount of phenols (p=2.2e-16) and scavenger activity of the free radical DPPH (p=2.2e-16). Also, we found a positive correlation between %DPPH scavenging activity and the total content of polyphenols (Pearson's coefficient=0.706) and a negative one between flavonoid (Pearson's coefficient=-0.485). The results suggest that in passionflower leaf samples the scavenging activity of the free radical DPPH is more related with polyphenols rather than flavonoids.

INTRODUCTION

Antioxidants are an important line of defense against free radical damage and are critical factors for maintaining optimum health and wellbeing conditions. Antioxidants are capable of stabilizing or deactivating free radicals before the latter attack biological targets in cells, so they are crucial for maintaining cumulative and debilitating oxidative stress [1]. The antioxidant activity

of natural products depends on the content of vitamin C, vitamin E, carotenoids, flavonoids and other polyphenols [2]. Different methodologies have been employed to evaluate the *in vitro* antioxidant activity of plants, including the use of free radical DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method [3,4]. In this regard, the antioxidant activity of some species of the genus Passiflora L. (PASSIFLORACEAE) has been studied by DPPH free radical scavenger activity [5].

Passionflowers are mostly herbaceous or woody vines, distributed in tropical and subtropical regions of the New World. About 500 species in the Americas and 30 species in South Asia and Oceania have been descried. In most recent taxonomic review, four subgenera, each with many sections, are distinguished by MacDougal and Feuillet [6]. Some cultivated species, particularly *P. edulis* Sim. (both, the yellow and the purple cultivars), *P. incarnata* L. and *P. alata* Curtis are recognizes as human food and phytomedicine and for this reason were introduced in many tropical countries. Today these plants are an integral part of phytopharmaceutical products all around the world and their preparation in tea like products is widely use in folk medicine since long time ago [5].

Main bioactive substances identified from the genus Passiflora include polyphenols and saponines, alkaloids, carotenoids, anthocyanins and sugars [7]. Polyphenols mainly belonging to the flavones C-glucoside class are present in common species such as *P. edulis* Sim, *P. incarnata* L and *P. alata* Curtis [7]. Isoorientin, a C-glucoside flavone found in *P. edulis* leaf extract was also found to be the major flavonoid in pulp extracts of this species [7,8]. In fact, the total flavonoid content in *P. edulis* pulp was reported to be quite significant in comparison with other beverages that are sources of flavonoids, such as orange juice [9].

Several flavonoids like apigenin, chrysin, orientin, vitexin, isovitexin, isoorientin and homorietin have been found in passionfruits (the fruits of passionflowers) [7-9]. By other hand, Passiflorine (and other glycosides) have been mostly found in leaf extracts [10]. In this regard, the biochemical composition of leaf extracts from most common passionflowers like *P. alata*, *P. incarnata* and *P. edulis* has been extensively studied over the past few decades, showing a predominance of alkaloids, saponins and mainly polyphenols [11-17]. However, phytochemistry of non common Passiflora species remain little explore and it's for this reason that we address our work at the comparison between less common and well documented species.

MATERIAS AND METHOD

In order to survey the available information about bioactive compounds of passionflowers the scientific databases EBSCOhost, EBM Reviews, BioMedCentral, ScienceDirect, Emerald, ISI Web of Science, Scielo and Google Scholar were consulted by a directed key word search for the following words: Passiflora, Phytochemistry, Pharmacological,Pharmaceutical, Clinical, Medicinal, Activity.

Plant Materials

Plant materials were obtained from live plants in vegetative phase (without the presence of flowers) as follows: *P. edulis, P. alata, P. incarnata, P. ligularis Juss, P. tripartita* (Juss) Poir. were collected in the garden of medicinal plants at the orchard of the Faculty of Agronomic Science (FCA) of the State University of Sao Paulo (UNESP), Botucatu in the State of Sao Paulo (Brazil); and samples of *P. coccinea* Aubl., *P. gardneri* Mast., *P. laurifolia* L. and *P. mucronata* Lam., were donated by the Agronomic Institute of Campinas (IAC), Sao Paulo, Brazil; All materials came from similar agro ecological conditions and were collected during the same week. The plant materials were air dried at 47 °C for 7 days. Dry leaves were separated from the stems, powdered, sifted and packed. Three experimental assays were performed with samples of the nine species using four repetitions for each one. The experiments were done at the Laboratory of Biochemistry, Institute of Biosciences at the Unesp-Botucatu.

Flavonoids

We followed the spectrophotometric method from Santos and Blatt [18] with Awad et al. [19] adjustment to compare the total amount of flavonoids between the studied species. Analyses of data were performed with the Levene´s test for homogeneity of variance followed by nonparametric Kruskal-Wallis test and Friedman test using Rstudio.

Polyphenols

To compare the total amount of polyphenols between Passiflora species, we followed the Folin-Ciocalteu spectrophotometric method in accordance with Singlenton et al. [20], as follows: 50 mg of dry sample were added to a solution of acetone 50%, shacked and submitted to an ultrasonic bath for 20 min and centrifugation for 10 min at 5000 rpm. Supernatant was collected and store at 2 °C in a dark glass container. The process was repeated to re-extract the sample and both supernatant were mixed. Experimental solutions were prepared with 0.1 ml of supernatant added to 0.9 ml of deionized water with 0.5 ml of Folin and 2.5 ml of sodium carbonate (20%), and then, absorbance was read at 725 nm. Data was statistical analyses by the Levene test for homogeneity of variance followed by one way Anova and the Last significance Difference (LSD) tests using Rstudio.

In vitro DPPH scavenger activity

In vitro free radical scavenging activity was measured using the radical chromogen 2,2-diphenyl-1-picrylhydrazyl (DPPH) photometric assay following Mensor, et al. [3]: 5 mg of dry sample from each of the studied species were extracted in 10 ml 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 [0.2 mg/ml], homogenized and leave in the dark. After 35 min, the samples were read in a spectrophotometer at 517 mm and compare with 3.5 ml of ethanol mixed with 300 µl of the same solution of DPPH. Data was statistical analyze by the Levene test for homogeneity of variance followed by one way Anova and the Last significance Difference (LSD) test. Also a correlation matrix between *in vitro* antioxidant activity and bioactive compounds (flavonoid and phenosl) was generated with Pearsos´s correlation coefficient using Rstudio.

RESULTS

In our bibliographic survey about passionflower's phytochemistry 176 scientific papers were found. Most studied species were *P. edulis* (62 papers), *P. incarnate* (53 papers) and *P. alata* (21 papers) with 82% of all publications. Other passionflowers, *P. ligularis*, *P. coccinea*, *P. laurifolia* and *P. tripartita* were mentioned only by occasional publications and we could not been able to find any works addressing the phytochemistry of *P. gardneri*. To us, it's important to notice that well documented species only represents 4% of the genus and the vast majority of passionflowers remains poor studied or biochemically unknown.

The total amount of flavonoids between the studied species of Passiflora did not have homogeneity of variance according to Levene's test (p=0.001). For this reason we conducted a non parametric Kruskal-Wallis (p=4.4e-5) and Friedman (p=0.25) tests which showed significant differences in the total content of flavonoids between the studied species (Table 1). Following Levene´s test (p=0.35) for phenols, we conducted an Anova test and found significant differences (p=2.2e-16) in the total amount of phenols (**Table 1**). A same analysis was performed to compare the *in vitro* DPPH scavenger activity (%DPPH); with Levene´s test (p=0.52) validating the Anova (2.2e-16) and the hypothesis of significant differences between the species. The results of the LSD test for the %DPPH are presented in **Table 1** and the correlation analyses of the variables are presented in **Table 2** and **Figures 1-3**.

Table 1. Comparison between bioactive compounds and *in vitro* antioxidant activities of nine species of Passiflora.

Species	Flavonoids g^{-1}100 g	Polyphenol g^{-1}100 g	DPPH %	TEAC µm/g sample
P. ligularis	0.002959cd	0.0036e	91.11a	9.63a
P. tripartita	0.002683ef	0.0080c	89.49a	9.89a
P. incarnata	0.003668b	0.0020g	37.67d	4.18d
P. alata	0.002449f	0.0019g	34.37d	3.85d
P. mucronata	0.0031c	0.0042d	59.64bc	6.67bc
P. gardneri	0.002819de	0.0021g	27.36d	3.13d
P. laurifolia	0.001825g	0.0117a	90.32a	10.05a
P. edulis	0.003905a	0.0025f	43.39cd	4.81cd
P. coccinea	0.001918g	0.0095b	91.76a	10.10a

Presented data is the mean value of four repetitions for each of the studied species of Passiflora. Vertical values followed by the same latter have not significant differences according with the LSD tests. Last significant difference: Flavonoids=0.00023698; Polyphenols=0.000340; %DPPH=21.22.

Table 2. Correlation matrix between bioactive compound and *in vitro* antioxidant activity.

	Flavonoids	Polyphenols	%DPPH
Flavonoids	1.0000	-0.738	-0.485
Polyphenols	-0.738	1.0000	0.706
%DPPH	-0.485	0.706	1.000

Values are Pearson's correlation coefficient for paired variables

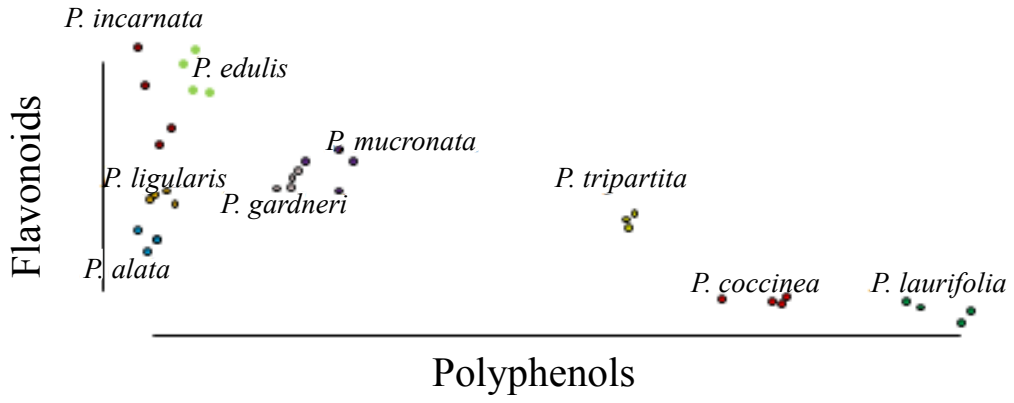

Figure 1. Correlations between the total content of flavonoids and polyphenols among the studied species of Passiflora.

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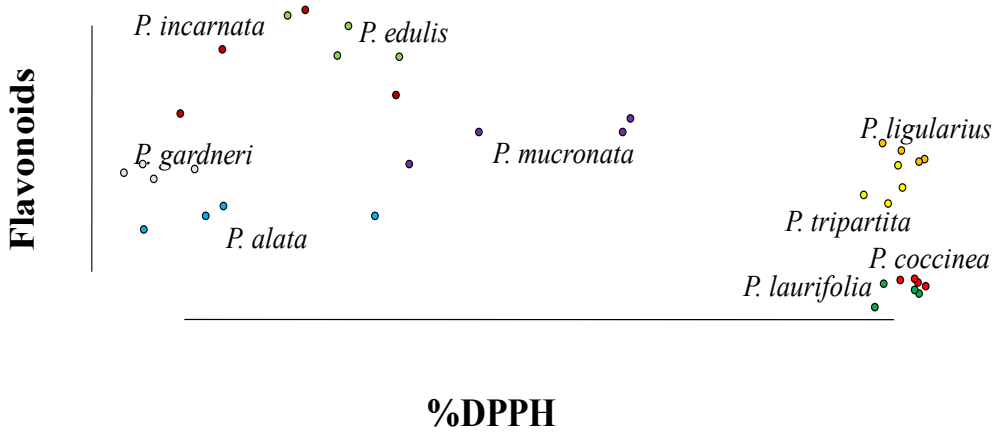

Figure 2. Correlations between the total content of polyphenols and the *in vitro* DPPH scavenger activity among the studied species.

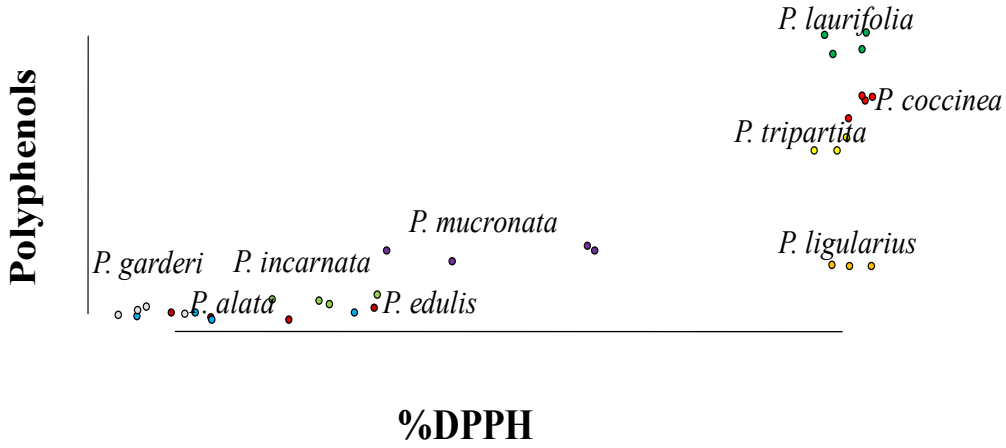

Figure 3. Correlations between the total content of flavonoids and the *in vitro* DPPH scavenger activity among the studied passion flowers.

DISCUSSION

According to Rudnick et al. many studies carried out over recent years have shown that polyphenols found in dietary and medicinal plants inhibit oxidative stress [21]. The leaf extract of passionflowers is rich in polyphenols, [14,16,17,21], but because Passiflora species differ in leaf chemistry, almost every species is unique with respect to the total amount and content of polyphenols and other bioactive compounds [22,23]. In this work we study the correlation between bioactive compounds and the *in vitro* antioxidant activity and compared the results between some well documented and poorly explored species including for the first time *P. gardneri* which doesn't have any previous report in scientific literature.

As in other research evaluating antioxidant activity of leaf extracts from medicinal plants,we found a direct linear relationship between the total polyphenols content and the *in vitro* antioxidant activity, indicating that polyphenols might be major contributors to the antioxidant activities of these extracts [21-24]. In their research Rudnick et al. found that P. alata showed a higher antioxidant activity when compared with *P. edulis*, but the antioxidant activities of both plants were significantly correlated with polyphenol contents [21]. In our results, *P. laurifolia* had more polyphenols (0.0117g-1100g) than any other passionflower and this was also correlated with high *in vitro* antioxidant activity (10.05 TEAC). However, *P. coccinea* with a little less polyphenos (0.0095g-1100g), presented the highest *in vitro* free radical DPPH scavenging activity (10.10 TEAC) and this could be probably explain by the presence of some polyphenols with higher antioxidant power, as Bendini et al. found with catechin and o-diphenol contents in P. nitida leaf extract. Moreover, same authors suggested that *P. foetida* leaf extracts, which showed high antimicrobial activity, had a low antioxidant activity because the low amounts of o-diphenol and catechin [23]. An interesting situation in our findings is the case of P. ligularis which showed low content of polyphenols but high *in vitro* antioxidant activity (Figure 3). In comparison, most popular species P. incarnata and *P. edulis* presented low *in vitro* DPPH scavenging activity, which could be explain by the reduced amount of polypenols (**Figure 3**). Furthermore and in accordance with the previously reported by several authors, these species showed the highest concentration of flavonoids between the studied passionflowers [10,17,23,25].

CONCLUSION

Several works in scientific literature showed that Passiflora species could be an important source of natural antioxidants but

regardless its high chemotaxonomic diversity only a few species have been broadly investigated. The vast majority of Passiflora species remains poor studied and more research should be conducted to elucidated Phytochemistry of this interesting plants.

Passiflora leaves content bioactive substances that are related with the *in vitro* DPPH scavenging activity. The total amount and contend of these substances can vary between Passiflora species and this variations influence the antioxidant activity. Thus we conclude that the scavenging activity of the free radical DPPH is more related with content polyphenols rather than flavonoids in passionflower leaf samples and by this mean Passiflora species with high content of polyphenols like *P. coccinea* have the strongest *in vitro* scavenging activity of the free radical DPPH.

REFERENCES

1. Atoui AK, et al. Tea and herbal infusions: their antioxidant activity and phenolic profile. Food Chem. (2005);89:27-36.
2. Saura Calixto F, Serrano J, and Goni I. Intake and bioaccessibility of total polyphenols in a whole diet. Food Chem. (2007);101:492-501.
3. Mensor LL, et al. Screening of brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. (2001);15:127-130.
4. Sánchez Moreno J, Larrauri JÁ and Saura-Calixto F. Free radical scavenging capacity of selected red, rose and white wines. J Sci Food Agr. (1999);79:1301-1304.
5. Montero DAV, et al. Comparation of free radical scavenging activity between seven species of *Passiflora*. Int J applied science and technology. (2014);4:208-211
6. MacDougal J, Feutllet C.Systematics. Ulmer T and MacDougal J (Eds.). Passiflora: Passion flowers of the world.Cambridge: Timber Press.27-31
7. Patel SS, et al. (2011) Recent updates on the genus *Passiflora*: A review. Int J Res Phytochem. Pharmacol. 1:1-16.
8. Dhawan K, Dhawan S, Sharma A (2004) Passiflora: a review update, J Ethnopharmacol. 94:1-23.
9. Zeraik ML and Yariwake JH. Quantification of isoorientin and total flavonoids in *Passiflora edulis* fruit pulp. Microchemical J. (2010);96:86-91.
10. Soulimani R, et al. Behavioural effects of *Passiflora incaranata* L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. J Ethnopharmacol. (1997);57:11-20.
11. Zanoli P, Avallone R and Baraldi M. Behavioral characterisation of the flavonoids apigenin and chrysin. Fitoterapia. (2000);S1:S117-S123
12. Antognoni F, Zhenga, S and Pagnucco C. Induction of flavonoid production by UV-B radiation in *Passiflora quadrangularis* callus cultures Fitoterapia. (2007);78:345-352.
13. Bombardelli E, et al. Passiflorine, a new glycoside from *Passiflora edulis*. Phytochemistry. (1975);14:2661-2665.
14. Yoshikawa K, et al. New cycloartane triterpenoids from *Passiflora edulis.* J Nat Prod. (2000);63:1377-1380.
15. Yoshikawa K, et al. Four cycloartane triterpenoids and six related saponins from *Passiflora edulis.* J Nat Prod. (2000);63:1229-1234.
16. Reginatto FH, et al. Steroidal and triterpenoidal glucosides from *Passiflora alata.* J Braz Chem. Soc. (2001);12:32-36.
17. Pereira CA, et al. A HPTLC densitometric determination of flavonoids from *Passiflora alata, P. edulis, P. incamata* and *P. caerulea* and comparison with HPLC method. Phytochem Analysis. (2004);15:241-248.
18. Santos MD and Blatt CTT. Teor de flavonóides e fenóis totais em folhas de P*yrostegia venusta* miers. de mata e de cerrado. Revista Brasileira de Botânica. (1998);21:135-140.
19. Awad AM, Jager AD and Westing LMV. Flavonoid and chlorogenic acid levels in apple fruit: characterization of variation, Scientia Horticulturae. (2000);83:249-263.
20. Singleton VL, Orthofer R and Lamuela-Raventos RM (1999).Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Packer, L (Ed.).Oxidants and Antioxidants, Part A, Methods in Enzimology, Academic Press, New York, 299:152-178.
21. Rudnicki M et al. Antioxidant and antiglycation properties of *Passiflora alata* and *Passiflora edulis* extracts. Food Chem. (2007);100(2):719-724.
22. Feuillet C, MacDougal J (2003) A new infrageneric classification of *Passiflora* L. (Passifloraceae). *Passiflora*, 13:34-38.
23. Bendini A et al. Phenol content related to antioxidant and antimicrobial activities of *Passiflora* spp. Extracts. Eur Food Res Technol. (2006);223:102-109.
24. Zainol MK, bd-Hamid A, Yusolf S, Muse R (2003). Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of *Centella asiatica* (L.) Urban. Food Chemistry. 81:575-581.

25. Petry RD et al. Comparative pharmacological study of hydroethanol extracts of *Passiflora alata* and *Passiflora edulis* leaves. Phytother Res. (2001);15:162-164.

26. Ulubelen A, Oksuz S and Mabry TJ. C-glucosylflavonoids from *Passiflora pittieri, P. alata, P. ambigua* and *Adenia mannii*. Journal of Natural Products. (1982);45:783.

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