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Extraction Parameters Affect Flavonoids Content and Antioxidant Activities in *Passiflora edulis*

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ABSTRACT

Passiflora edulis Sims (Passifloraceae) is a widely distributed species ranging from tropical and sub-tropical regions of the world. It has been commonly used as a food and possesses various medicinal properties. This research aims to see the effect of different extraction methods and drug/solvent ratio on phenolics and flavonoids content as well as the antioxidant capacity in P. edulis leaves. The powdered leaves of P. edulis were extracted with hydroalcoholic solution employing maceration, percolation and Sohxlet extraction and three drug/solvent ratios (1:8, 1:10; 1:12). The total phenolics and flavonoids contents of different extracts were determined by Folin-Ciocalteu and colorimetric $AlCl_3$ methods, respectively. Antioxidant activity was investigated by using in vitro DPPH and FRAP assays. Flavonoids were identified by LC-MS/MS on the basis of mass spectral analysis. The highest content of phenolics (16.30%), flavonoids (3.88%) and antioxidant activity (DPPH IC_{50} 84.23 µg ml^{-1}; FRAP value 1.89 mmol Fe^{2+} g^{-1}) were obtained with maceration at drug/solvent ratio of 1:8. Six C-glycosyl flavones and one O-glycosyl flavone were tentatively identified. A correlation was demonstrated between the total phenolics and flavonoids content, and antioxidant activity. The results obtained here provide a useful technique to extract the natural substances from P. edulis that may serve as a potential source of antioxidants from natural origin.

Keywords: Extraction method; Medicinal plant; Phenolic compounds; LC-MS/MS; Passifloraceae

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INTRODUCTION

The genus *Passiflora* L. is one of the largest genus of the family Passifloraceae, comprising about 520 species distributed worldwide [1]. *Passiflora edulis*, commonly called as passion fruit or maracujá, is the most popular among them. This plant species is native to Brazil, which is the largest consumer and producer of passion fruit in the world [2]. It is also cultivated in other countries for its edible fruit and presents ornamental and pharmaceutical interest. The leaves of this plant species have been widely used in folk medicine due to pharmacological properties, such as sedative or tranquillizer [3, 4]. *P. edulis* is known to possess several pharmacological effects, such as central nervous system depressant [5, 6], anxiolytic [7, 8, 9], antimicrobial [10, 11], antitumor [12], anti-inflammatory [13, 14], anti-hypertensive [15], and antioxidant [16, 17, 18]. These properties are attributed to the presence of bioactive compounds, mainly flavonoids in this plant species.

Phytochemical studies have shown the presence of cyanogenic glycosides [19, 20], alkaloids [5, 21], triterpenes [6, 22, 23] and flavonoids [24] in various parts of this plant species. Specifically, in relation to flavonoids, apigenin and luteolin glycosides derivatives have been characterized, including *C*-glycosides (orientin, isoorientin, vitexin, isovitexin) [24, 25] and *O*-glycosides flavonoids derivatives (luteolin-7-O-[2-rhamnosylglucoside]) [8].

In addition, *P. edulis* has been included in the list (prepared by the Brazilian Ministry of Health) of medicinal plants having potential to generate functional and pharmacological products [26]. In this regards, it is important

to ensure the quality of raw materials and their derivatives products, which can be achieved through the evaluation of chemical constituents in the quality control analysis.

The selection of suitable extraction method is one of the main requirements for characterization of bioactive compounds from plant material. The most common factors affecting the extraction processes are solvent, temperature, pressure, time and plant characteristics, so it might be necessary to use various conditions or extraction procedures for proper extraction of chemical markers, which will determine the quality and effectiveness of herbal medicines from plants [27, 28].

Considering that plant extracts represent the most commonly used preparations in herbal formulations; involving operational stages with several variables that may change the stability of the chemical constituents and their therapeutic activities. Therefore, it is essential to determine the optimized set of parameters for the extraction of chemical markers from plant species in order to prove safety, efficacy and quality [29].

Currently there is a demand for standardized herbal extracts in order to be used as a chemical marker or to check the authenticity of material. There are only a few reports on leaves of *Passiflora* sp. describing the standardization of its extracts. Oliveira et al. [30] studied the spouted-bed and spray drying performance to see their efficacy on standardized dried leaf extracts of three Brazilian plant species including *P. alata*. Another study evaluated the effect of extraction conditions on total phenolics content and antioxidant activity in passion fruit peel [31].

The aim of the study was to investigate the influence of extraction procedures affecting the flavonoid extraction, phenolic content as well as antioxidant activity in *P. edulis* leaves. The present study would be helpful in development of *P. edulis* as a phytopharmaceuticals as well as in cosmeceutical preparations. Further, antioxidant compounds, such as flavonoids identified from this plant species can help in protection against various diseases in which the oxidative species are involved and it may further lead to the search for a new natural product with antioxidant properties.

EXPERIMENTAL SECTION

Collection and botanical identification

Leaves of *Passiflora edulis* Sims were collected at the city of Paço do Lumiar, Maranhão State, Brazil (2°30'9" S; 44°9'27" W), in January 2013. The voucher specimens were deposited in the Ático Seabra Herbarium (SLS), of the Federal University of Maranhão, under the number of 1155/SLS017213, and were authenticated by Ana Zélia Silva.

Preparation of *P. edulis* extracts

Leaves of *P. edulis* were dried at 40° C in an oven with circulating air and powdered with a knife mill to obtain a moderately coarse powder. In brief, the extracts of *P. edulis* were obtained using factorial design: extraction process and hydromodule (drug/solvent ratio). The powder of *P. edulis* leaves (50g) were extracted with 70% ethanol, separately, by maceration (M), percolation (P) and in a Soxhlet (S) apparatus, using 1:8, 1:10 and 1:12 drug/solvent ratio. Each extractive solution was concentrated to a small volume at 40 °C in a rotary evaporator under vacuum, to obtain the hydroalcoholic extracts of *P. edulis* (M1:8, M1:10, M1:12, P1:8, P1:10, P1:12, S1:8, S1:10 and S1:12 respectively).

Total Phenolic Content (TPC)

The TPC of all samples was determined with the Folin-Ciocalteu reagent and 20% sodium carbonate. The reaction mixture was kept in the dark for 2 h at room temperature and absorbance was then measured at 760 nm in a Lambda 35 UV-vis spectrophotometer (Perkin Elmer, Inc., Waltham, MA, USA) [32]. TPC was calculated from the calibration curve constructed with standard solutions of gallic acid (1.0-30.0 μg ml^{-1}) and is expressed as gallic acid equivalent (%).

Total Flavonoid Content (TFC)

The TFC of all samples was determined with methanolic solution of aluminum chloride ($AlCl_3$) 5%. The reaction mixture was kept in the dark for 30 min at room temperature and absorbance was then measured at 425 nm in a Lambda 35 UV-vis spectrophotometer (Perkin Elmer, Inc.) [33]. TFC was calculated from the calibration curve constructed with standard solutions of quercetin (1.0-30.0 μg ml^{-1}) and is expressed as quercetin equivalent (%).

DPPH Radical scavenging activity

The antioxidant activity of the samples of *P. edulis* was evaluated by using the DPPH free radical scavenging assay as already described by Brand-Williams et al. [34] with some modifications. The samples were diluted in methanol at different concentrations (1.0-100.0 μg ml^{-1}) and added to a methanol solution of DPPH (40.0 μg ml^{-1}). After 30 min of reaction at room temperature in the dark, the absorbance of each solution was read at 517 nm

in a Lambda 35 UV-vis spectrophotometer (Perkin Elmer, Inc.). Standard of Trolox® was treated under the same conditions as the samples. The percent inhibition was calculated using the formula:

$$\text{DPPH}_{\text{scavenging activity}}\ (\%) = 100 - [(A_{\text{sample}} - A_{\text{blank}}) \times 100/A_{\text{control}}],$$

Where A_{sample} = absorbance of the sample after 30 min of reaction, A_{blank} = absorbance of the blank, and A_{control} = absorbance of the control.

The percentage of scavenging activity was plotted against the sample concentration to obtain the IC_{50}, defined as the concentration of sample necessary to cause 50% inhibition. All experiments were done in triplicate.

Ferric Reducing Antioxidant Power Assay (FRAP)

The method described by Benzie and Strain [35], with some modifications, was used to determine the antioxidant activity based on iron reduction using the FRAP assay. FRAP measures the ferric-reducing ability of a sample in acidic medium (pH 3.6), forming an intense blue color as the ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex, which is reduced to the ferrous (Fe^{2+}) form. FRAP reagent was prepared immediately before analysis by mixing 25 ml of acetate buffer (300 mM, pH 3.6), 2.5 ml of TPTZ solution (10 mM TPTZ in 40 mM HCl), and 2.5 ml of $FeCl_3.6\ H_2O$ (20 mM) in aqueous solution. Different concentrations of 100 µl of the samples (1–100 µg/ml) were added to 300 µl of distilled water and 3.0 ml FRAP reagent, and the mixtures were incubated in a water bath at 37°C for 30 min. The absorbance of the reaction mixture was read at 593 nm in a Lambda 35 UV-vis spectrophotometer (Perkin Elmer, Inc.) using FRAP solution as a blank. The calibration curve was drawn using different concentrations of $FeSO_4.7H_2O$ (0–2000 µM) ($r^2 = 0.9987$) and the results are expressed as millimoles of Fe^{2+} per gram of sample. Standard of Trolox® was treated under the same conditions as the samples.

UV/vis analysis

The UV spectra were recorded in triplicate from 200 to 600 nm with a Lambda 35 UV-vis spectrophotometer (Perkin Elmer, Inc.). Quartz cells (1 cm) were used for absorbance measurements.

HPLC/UV-vis analysis

HPLC analysis was carried out in a Thermo Finnigan Surveyor Autosampler liquid chromatograph (San Jose, CA, USA) equipped with an injector with a 25-µL loop and a UV detector. A Hypersil BDS C-18 column (250 x 4.6 mm, 5 µm; Thermo Electron Corporation, Waltham, MA, USA) was used. The compounds from *P. edulis* extracts were separated at room temperature using a gradient elution program at a flow rate of 1.0 ml/min. The mobile phases consisted of purified water containing 0.1% formic acid (A) and acetonitrile (B). The injection volume into the HPLC system was 25 µL and UV-vis detection was performed at 254 nm.

LC-MS/MS analysis

The M1:8 were analyzed with an HPLC system (CBM-20A, Shimadzu) equipped with a UV/vis detector which was coupled to an Esquire 3000 Plus ion-trap mass spectrometer (Bruker Daltonics, Bremen, Germany) using electrospray ionization (ESI). The mobile phase composition was the same as described above. The ionization conditions were adjusted as follows: electrospray voltage of the ion source of 40 eV, capillary voltage of 4.0 kV, and capillary temperature of 320°C. Ultrahigh pure helium (He) was used as the collision gas and high-purity nitrogen (N_2) as the nebulizing gas. Nebulization was aided with a coaxial nitrogen sheath gas provided at a pressure of 27 psi. Desolvation was facilitated using a counter current nitrogen flow set at a rate of 7.0 L/min. Analysis were carried out using full-scan mass spectra in the negative ionization mode and data-dependent MS^2 scans from *m/z* 100 to 3000. The compounds were tentatively identified on the basis of the molecular ion mass, fragmentation and compared to those described in the literature.

Statistical analysis

All analysis were performed in triplicates. The results are expressed as the mean ± standard deviation and were analyzed using the GraphPad Prism 5.0 program. One-way analysis of variance (ANOVA) and Tukey's multiple comparisons test were used to determine significant differences between means. A level of significance of $p < 0.05$ was adopted. Pearson's correlation test was used to evaluate the correlation between TPC (%), TFC (%), DPPH free radical-scavenging activity (IC_{50}), and ferric-reducing ability (mmol $Fe^{2+}g^{-1}$sample).

RESULTS AND DISCUSSION

Total phenolic, flavonoid content and antioxidant activity

Extraction is a key step involved in obtaining bioactive compounds from plants. Different extraction procedures have been employed to extract the compounds from plants. Concentration, quality as well as biological activities of plant extract showed significant variations depending on the extraction method used [36]. Therefore, it is quite important to select the suitable extraction method for obtaining plant extract with optimum concentration

and activities. In the present study, three extraction methods, namely maceration (M), percolation (P) and Sohxlet (S) along with three drug/solvent ratios (1:8, 1:10 and 1:12) have been used to see their efficacy on extraction of chemical constituents and antioxidant activities in *P. edulis*. Among the different extraction and solvent methods, M in 1:8 drug/solvent ratio provided the best results as compared to P or S (in all the drug/solvent ratios) (Table 1). The results of the *P. edulis* extracts obtained by analysis of total phenolic (TPC), and total flavonoid contents (TFC) as well as antioxidant capacity as estimated by the radical-scavenging activities in the DPPH (2,2-diphenyl-1-picrylhydrazyl) and by the ferric reducing ability power (FRAP) using the ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex are presented in Table 1.

Table 1: Total phenolics and flavonoids content, and antioxidant activity in the extracts of *Passiflora edulis*

Sample	TPC (%)	TFC (%)	DPPH	FRAP value
			(IC_{50} µg ml^{-1})	(mmol Fe^{2+} g^{-1})
M1:8	16.30 ± 0.32^{a}	3.88 ± 0.04^{a}	84.23 ± 0.77^{a}	1.89 ± 0.06^{a}
M1:10	11.32 ± 0.13^{b}	2.49 ± 0.01^{b}	99.92 ± 0.91^{b}	1.02 ± 0.01^{b}
M1:12	14.91 ± 0.36^{c}	3.37 ± 0.01^{c}	$90.27 \pm 3.02^{a,c}$	1.55 ± 0.05^{c}
P1:8	13.79 ± 0.04^{d}	2.94 ± 0.01^{d}	$94.77 \pm 0.96^{b,c}$	$1.43 \pm 0.11^{c,d}$
P1:10	13.09 ± 0.07^{e}	2.90 ± 0.35^{e}	$97.37 \pm 0.40^{b,c}$	$1.24 \pm 0.03^{b,e}$
P1:12	11.98 ± 0.06^{f}	2.58 ± 0.01^{f}	$97.75 \pm 1.06^{b,d}$	1.12 ± 0.02^{b}
S1:8	12.21 ± 0.04^{f}	3.34 ± 0.01^{g}	$97.61 \pm 3.83^{b,e}$	1.15 ± 0.01^{b}
S1:10	13.93 ± 0.10^{d}	4.11 ± 0.01^{a}	$92.39 \pm 5.23^{c,d,e}$	1.45 ± 0.02^{c}
S1:12	13.77 ± 0.16^{d}	3.35 ± 0.01^{g}	88.69 ± 1.73^{a}	$1.30 \pm 0.08^{d,e}$
Trolox®	-	-	5.11 ± 0.04^{f}	9.09 ± 0.10^{f}
Different letters indicate significant differences ($p < 0.05$; One way ANOVA; Tukey's test)				

The TFC and TPC values of extract tested with different methods and drug: solvent ratios were found to ranged between 2.49 - 4.11% and 11.32 - 16.30%, respectively. The M1:8 extract showed the highest value of TPC (16,30%) followed by M1:12 (14,91%), while TFC values were found to be higher in M1:8 (3,88%) and S1:10 (4,11%) extracts. Maceration and Soxhlet extraction methods are versatile, relatively simple, safe and inexpensive and thus have been followed for efficient extraction of bioactive compounds from several plant species including *Azadirachta indica*, *Cynara scolymus*, *Punica granatum*, *Salacia lehmbachii* and *Spondias mombin* [28, 37, 38, 39, 40, 41].

Earlier studies with *P. edulis* (collected in southern Brazil) showed the TFC of 4.60% (w/w) in hydroalcoholic extract obtained by extraction at reflux [7]. In literature aqueous extracts of leaves of *P. edulis* obtained by reflux, reported TFC of 4.04% (w/w) [42] and 3.9% (w/w) [4]. The synthesis of secondary metabolites in plants is the result of interaction with the environment. Thus, the difference of quantitative chemical composition of flavonoids in plant sample collected from different regions could be due to various extrinsic factors, such as time, weather and place of collection [43].

A stable free radical, DPPH has been used to estimate the free radical-scavenging activities of plant extracts. In the analysis of DPPH, a lower IC_{50} value indicates greater antioxidant activity, as a smaller quantity of extract is required to inhibit 50% of the DPPH radical. The results obtained during present investigation showed that extract obtained by maceration in a lower drug/solvent ratio (M1:8) are able to significantly (IC_{50} value 84.23 µg ml^{-1}) reduce *in vitro* DPPH. concentration and hence imply strong antioxidant activity. Earlier studies by Sunitha and Devaki [44] have also reported the antioxidant potential of ethanolic extract of *P. edulis* leaves with DPPH IC_{50} of 875 µg ml^{-1}. The antioxidant potentials of the *P. edulis* extracts were also estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) (FRAP assay). The extract obtained with M1:8 showed the highest ferric-reducing ability (1.89 mmol Fe^{2+} g^{-1}) as compared to P and S extraction method with all the drug solvent ratios.

However, aqueous extracts of leaves of *P. edulis*, have showed the values 128 µg ml^{-1} [25] and 1100 µg ml^{-1} [18]. Some studies have shown that binary solvent system, especially water alcohol mixture was more effective than mono-solvent system in extracting the antioxidant compounds as observed in mention plant species [31, 45, 46].

In the present study, high antioxidant activity was observed in M1:8, both by DPPH radical scavenging and FRAP assays. Likewise, this extract had the highest contents of TPC and TFC. Several studies have indicated a

relationship between the antioxidant activity and contents of phenolic compounds, such as the flavonoids [17, 47, 48, 49].

Phenolic compounds have been highlighted as important antioxidant substances of natural origin, especially by inhibiting the process of lipid peroxidation in cell membranes. The chemical properties of polyphenols in terms of availability of phenolic hydrogens to act as hydrogen donor, reducing agents of singlet oxygen predicts their antioxidant activity [50].

The Pearson's correlation coefficients between TPC and TFC as well as DPPH and FRAP assays are presented in Figure 1. A negative correlation was observed between the DPPH–FRAP (–0.8911), DPPH–flavonoid content (–0.7358), DPPH–phenolic content (–0.9288), whereas in the DPPH assay a low IC_{50} value has relation with high contents of phenolic compounds and flavonoid, and a high value of FRAP [51]. However, a positive correlation was found between FRAP–TPC (0.9803), FRAP–flavonoid (0.7259), and TPC–TFC (0.7152). These findings reveal that the reducing power by FRAP assay could be presence of phenolic compounds, and that the content of phenolic compounds is due to flavonoids. These results suggest that total phenols, particularly flavonoids, present in the extracts of *P. edulis* are mainly responsible for the antioxidant activity.

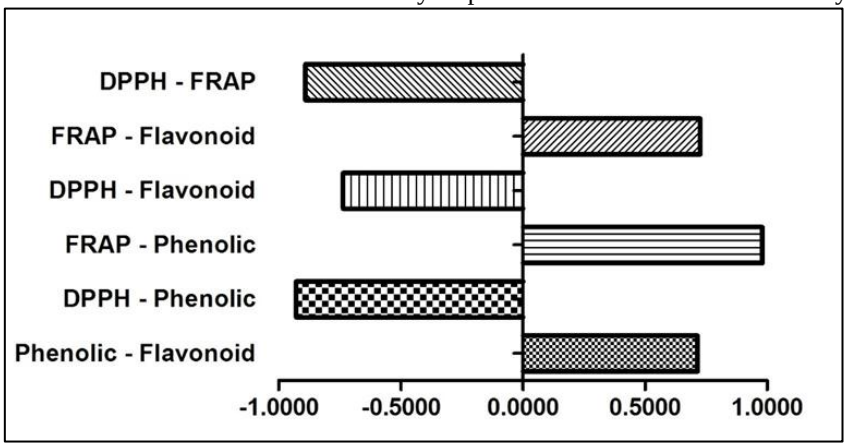

Figure 1: Pearson correlation coefficient between the total phenolic content, total flavonoid content, and DPPH and FRAP methods

In general, a higher TPC value gave a stronger antioxidant activity. Some authors have mentioned this relationship [32, 52, 53] and described important antioxidant activity of *P. edulis*, which has been correlated with the content of phenolic compounds [49, 54, 55]. It has been reported that the antioxidant activity of the extract from *P. edulis* (0.23 ± 0.02 TEAC) is significantly correlated with the content of polyphenols (92.5 ± 2.2 μg mg^{-1} of extract) [16].

The extraction procedure is an important step in the study of bioactive constituents from medicinal plants. Which the main purpose is to separate plant metabolites from insoluble residue, giving a complex mixture of metabolites. Therefore, among the commonly used extraction methods, maceration is a simple, popular and inexpensive way to obtain bioactive compounds [28, 56], such as phenolics. The most common methods of phenolic extraction employ organic solvents such as methanol, ethanol, acetone, ethyl acetate [57] and their combinations, often with different proportions of water. Our results suggest that the leaf extract of *P. edulis* obtained by maceration with hydroalcoholic solution could be a good choice of natural compounds with antioxidant activities.

UV-vis Spectra and chromatographic profile of *P. edulis* extracts

In the analysis of *P. edulis*, all samples showed similar UV spectra and HPLC profiles. The UV-vis spectra obtained for the extracts exhibited maximum absorption wavelength at 271 and 333 nm. The typical UV-vis spectra of flavones include Band A in the 310-350 nm range and Band B in the 250-290 nm range [58]. Therefore, these data suggest that compounds in extracts of *P. edulis* are flavonoids, and these in turn are the class of flavones. Flavonoids are reported to be the major phytoconstituents of the genus *Passiflora* [59], and has been shown to be rich sources of *C*-glycosyl flavones [25].

Despite the variation of peak intensity, the chromatographic profiles of the nine extracts from *P. edulis* leaves were similar (Figure 2).

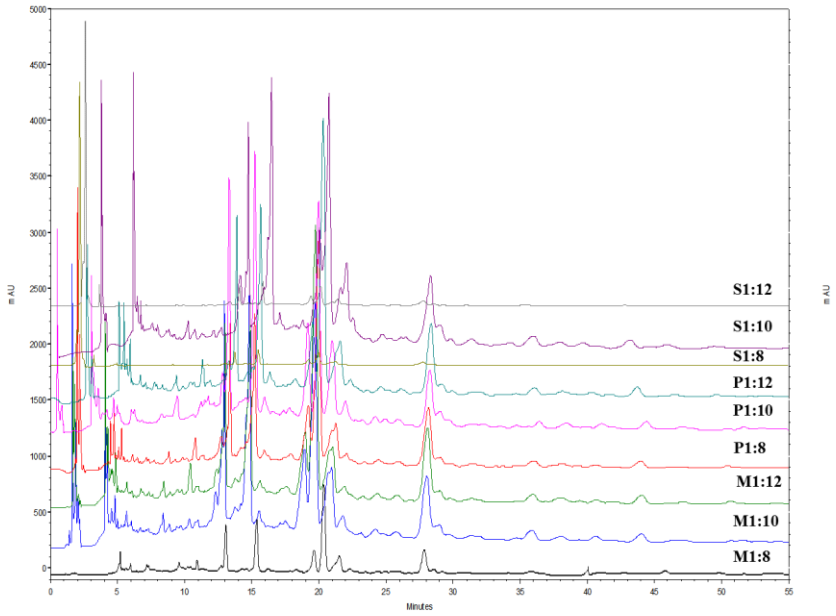

Figure 2: Characteristic chromatographic profiles of extracts of *Passiflora edulis*

Chemical fingerprints obtained by spectral and chromatographic techniques represent an important tool for quality control of herbal medicines and its products. It demonstrates the both sameness and differences between various samples. HPLC fingerprint is considered a useful approach to identification and authentication of plant species and their extracts [60].

Chemical composition of M1:8 by LC-MS/MS

Since, M1:8 had higher TPC, and was more effective against the DPPH radical and FRAP assay, the chemical composition of this sample was analyzed by LC-MS/MS, identification was based on fragmentary ions *m/z* compared to those described in the literature.

In this study, we tentatively identified seven constituents (compounds 1-7) (Figure 3). Their retention time, molecular weight, molecular ion $[M - H]^-$, and main product ions obtained by LC-MS/MS for the fragmentation peaks of hydroalcoholic extract of *Passiflora edulis* leaves are summarized in Table 2.

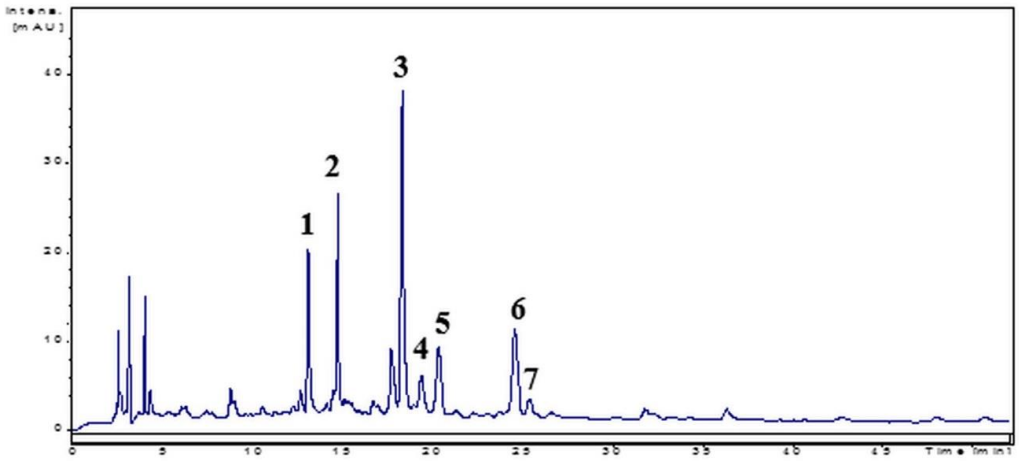

Figure 3: HPLC chromatogram of compounds detected in the hydroalcoholic extract obtained by maceration, 1:8 drug/solvent ratio (M1:8) of *Passiflora edulis* leaves. Peak numbers correspond to the compounds tentatively identified shown in the Table 2.

Table 2: Tentative identification of flavonoids compounds in extract of *Passiflora edulis* leaves (M1:8) by LC-MS/MS data

Compound no.	t_R (min)	MW	$[M - H]^-$ (*m/z*)	MS/MS (*m/z*)	Tentative identification	Reference
1	13,1	610	609	519, 489, 369	luteolin-6-*C*-hexoside-8-*C*-hexoside (lucenin-2)	[61, 62, 63]
2	14,7	594	593	575, 503, 473, 383, 353	apigenin 6-*C*-hexoside-8-*C*-hexoside (vicenin-2)	[61, 63, 64]
3	17,7	564	563	545, 503, 473, 443, 383, 353	apigenin 6-*C*-pentoside-8-*C*-hexoside (isoschaftoside)	[61, 65]
4	18,4	448	894	447	luteolin 6-*C*-hexoside (isoorientin)	[61, 66
5	20,3	578	577	559, 487, 457, 367	vitexin 2”-*O*-rhamnoside	[67, 68]
6	24,7	432	431	341, 311	apigenin 8-*C*-hexoside (vitexin)	[62, 69]
7	25,4	594	593	475, 391, 285	luteolin-7-*O*-rutinoside (scolymoside)	[70, 71, 72]

***t*R, Retention time; MW, molecular weight; $[M - H]^-$ molecular ion**

The use of hyphenated techniques provided indicative data for structural determination of the compounds present in the extract of *P. edulis*, as confirmed with literature data. It was possible to identify three 6,8-di-*C*-glycosyl flavones (vicenin-2, lucenin-2 and isoschaftoside), two mono *C*-glycosyl flavones (vitexin and isoorientin) frequently reported for *Passiflora* species, one *O*-diglycosyl flavone (luteolin 7-*O*-rutinoside) and a *C*-diglycosyl flavone (vitexin-2"-*O*-rhamnoside), as far as we know, vitexin-2"-*O*-rhamnoside is detected for the first time in *P. edulis* leaves in the present study.

Flavonoids and their glycosides has gaining importance in quality control of herbal medicines and medicinal plants and the application of mass spectrophotometry seems to be a valuable technique for structural analysis of these compounds in plant extracts [73, 75]. Whereas the marker is selected as a characteristic component of the plant, the *C*-glycosyl flavonoids, which prevail in the leaves of *P. edulis*, could be used as chemical markers for the standardization of products derived from this plant species.

A positive correlation was observed among all the parameters analyzed i.e. phenolic and flavonoid contents, DPPH and FRAP. Furthermore, the M1:8 extract exhibited the best results during the analysis, demonstrating that the process of extraction by maceration, in a drug/solvent ratio of 1:8 can be considered as a good option for the extraction of flavonoids, having antioxidant activity. Therefore, the antioxidant activity could be due to the synergistic interactions between these flavonoids.

CONCLUSIONS

The knowledge of the chemical composition of *P. edulis* is essential for standardization of extraction parameters, relevant tool in contributing to safety, efficacy and high quality of products derived from species to be developed by the pharmaceutical industries. With regard to the extraction method the best results were obtained with maceration at drug/solvent ratio of 1:8. Here, we used HPLC combined with MS to study the phenolic constituents of *P. edulis*. The results revealed the presence of several flavones in extract of *P. edulis* leaves with moderate antioxidant activity, which shows that *P. edulis* may serve as an additional natural source of bioactive compounds. In conclusion, we suggest that *P. edulis* could be considered as one of the natural antioxidant sources and dietary nutritional supplements to prevent oxidation-related diseases.

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