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Comparative Evaluation of Chemical Compositions and Antioxidant Activities of *Passiflora edulis* Sims Extracts

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ABSTRACT

Passiflora members have an important quality in both conventional and modern medicine. In the present study *Passiflora edulis* Sims from MediterraneanTurkey (Antalya-Gazipaşa) was investigated from the aspects of phenolic compounds and antioxidant activities The plant material (stems, leaves) used as a whole and chloroform, ethyl acetate and methanol were used for the extraction. High performance liquid chromatography was used to analyse the phenolic compounds of the extracts. Besides, the antioxidant potential of the extracts was characterised by the total phenolic content, the total flavonoid content, the DPPH (2,2-diphenyl-1-picrylhydrazyl), the CUPRAC (Cupric Ion Reducing Antioxidant Capacity) and the FRAP (ferric reducing antioxidant power assay) tests. Methanol extract was found to be more active than the other extracts in the all antioxidant experiment. DPPH radical scavenging activity of methanol extract was 0.028 ± 0.001 mg/mL, FPAP assay was 0.511 ± 0.012 µmolGA/g and CUPRAC assay was 3.728 ± 0.150 µmolGA/g. As a result, methanol extract of *Passiflora edulis* was established ferulic acid (175.12 mg std/g) and *p*-coumaric acid (116.88 mg std/g) were determined as primary phenolic compounds in different amounts along with protocatechuic acid, chlorogenic acid, caffeic acid, apigenin and isorhanmetin. The results showed that *Passiflora edulis* deserves to be studied both for its use as a food and for its therapeutic properties.

Keywords: Antioxidant activity, HPLC-DAD, *Passiflora edulis*, Turkey.

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Introduction

Reactive oxygen species (ROS) play an important role in human diseases and disorders. Accordingly, ROS level/concentration is an important factor in the growth, progression and establishment of various diseases [1]. In a normal healthy cell, ROS in cells are balanced within the cell by the antioxidant system, which is a variety of enzymatic and non-enzymatic compounds. However, it is also known that in disease or pathological conditions, ROS levels increase, antioxidants are depleted and ultimately oxidative stress causes deterioration. Antioxidant supplementation is important to reduce oxidative stress and therefore related diseases [2-4].

Passiflora belongs to the family of Passifloraceae, comprises approximately 600 species distributed in the tropical and subtropical regions around the world [5-6] Passiflora species occupy an important place in both conventional and modern medicine;. they are good source of biologically active substances containing antioxidants and immune modulators with tremendous therapeutic potential [7-9]. Various phytochemical investigations have revealed the presence of secondary metabolite among them phenolics, flavonoids, alkaloids, saponins and terpens [8-14], for Passiflora species.

Moreover, this species is also known to posses many biological activities including anticancer, antioxidant, antiproliperative, sedative, antihypertensive, analgesic [7,10,13,15-17]. Extracts of leaves of different species of Passiflora plant are used in traditional medicine for the treatment of disorders of the nervous system such as migraine and insomnia, anxiolytic, whooping cough, bronchitis and asthma, and also as sedative, anticonvulsant and analgesic [6,8,19].

Passiflora edulis has been studied for its antimicrobial, anticancer and antioxidant potential. However, studies on phytochemical content and antioxidant activity of the plant in solvents with different polarity are limited. Therefore, with this study was conducted for two purposes: (1) to identify and quantify phenolic compounds in the chloroform, methanol and ethyl acetate extracts of *P. edulis* using HPLC-DAD, (2) to evaluate the antioxidant activity of the extracts using 2,2-diphenyl-1-picrylhydrazil (DPPH), FRAP and CUPRAC, total phenolic content and total flavonoid content antioxidant tests.

Materials and Methods

Chemicals and Solvents

The chemicals and reagents, ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), neocuproin, $CuCl_2$, 2,4,6-Tris(2-pyridyl)-s-triazine, Iron (III) Chloride Hexahydrate, Acetic Acid Glacial, HCl, Sodium Hydroxide, were purchased from Merck (Darmstadt, Germany) and Aldrich (Milwaukee, WI, USA). All chemicals were used in analytical pure form. HPLC-DAD analyses was performed using HPLC grade phenolic standards and solutions.

Sample Preparation

Passiflora edulis was collected from Antalya-Gazipaşa, in Türkiye the coordinates of 36°16'0.428"N, 32°19'37.674". The collected plant specimens were identified by Vagıf Atamov at the Biology Department of RTE University. The plant material (stems, leaves) used as a whole were dried in the shade at room temperature. They were then ground in a blender. Three different solvents were used for extraction. Five grams of the dried sample were put into three flasks and extracted with 50 mL of chloroform, ethyl acetate and methanol in an ultrasonic bath (Heidolph, Germany) at 40 °C for 60 min. Each extract was centrifuged at 10000 rpm, for 10 min. The extracts were transferred into new flasks and evaporated using a rotary evaporator. The dried extracts were dissolved in methanol. The solution was stored at -18 °C until analysed. The extraction procedure was modified according to the method developed by Selvi et al. (2018) [19].

Methanol the most preferred solvents for phenolic compounds because the polyphenols are mostly well soluble and stable in methanol and ethanol [20-23]. While chloroform is preferred for non-polar compounds, ethyl acetate is mostly suitable for flavonoid extraction [24]. Polyphenols in plants have a wide polarity range and the use of solvents with different polarities in extraction is more suitable for the extraction of phenolic compounds. Therefore, extraction was performed using three extraction solvents to elucidate the phenolic content of *P. edulis*.

Determination of Phenolic Compounds by HPLC-DAD

HPLC analyses were performed with Thermo Ultimate 3000 series HPLC system. Chromatographic separation was performed on Agilent C18 column (4.6 × 150 mm, 5 µm) by using a gradient elution at a flow rate of 1 mL/min, the column temperature was 30 °C and injection volume was 20 µL. Gradient elution was used for HPLC analyses using Selvi et al (2024) [25]. Detection wavelengths were set at 280 and 315 nm. Gallic acid, protocatechuic acid, catechin, chlorogenic acid (3-caffeoylquinic acid), caffeine, caffeic acid, vanillic acid, rutin, p-coumaric acid, ferulic acid, o-coumaric acid, quercetin, apigenin, kaempferol, and isorhamnetin were used for phenolic standards.

Determination of Total Phenolic Content (TPC)

TPC of the extracts of *P. edulis* were analysed with Folin-Ciocalteu's phenol reagent. Gallic acid was used to generate a standard curve in a range from 0.0020 and 1.00 $mg.mL^{-1}$ ($r^2 = 0.999$) [26]. All the experiments were carried out in triplicate and the absorbance of the mixture was measured using a UV-Vis spectrophotometer at 760 nm (Labomed Inc. Culver City-USA). The concentrations of total phenolic compounds were given as mg of gallic acid equivalent (GAE) per g of dry weight (dw).

Determination of Total Flavonoid Contents (TFC)

The aluminum complexation method, as reported by Marcucci *et al.* (1998) [27], was used to determine the total flavonoid concentration. Using this procedure, 0.5 mL of plant extract was mixed with 0.1 mL of 10% aluminum nitrate, 0.1 mL of 1 M potassium acetate, and 4.3 mL of 80% ethyl alcohol. After 40 minutes of room temperature incubation, the samples were subjected to UV-Vis (Labomed Inc., Culver City, USA) absorbance measurement at 415 nm. Using quercetin as the standard, a calibration curve in the range of 0.00195 to 0.5 $mg.mL^{-1}$ ($r^2 = 0.999$) was produced. The total flavonoid content was represented as mg of quercetin equivalent (QE) per g of dry weight (dw), based on the mean of three readings.

Free Radical Scavenging Activity Assay (DPPH)

The scavenging activity of the extracts against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by spectrophotometric method at 517 nm [28]. Extracts were prepared at 2.0 mg/mL concentrations and diluted to different concentrations in methanol in the range of 1.00-0.025 mg/mL. Briefly, 0.75 mL of 0.1 mM DPPH in methanol was added to 0.75 mL of plant extract. Gallic acid and quercetin were used as standards to measure radical scavenging activity. Results were expressed as SC_{50} values, which indicate the sample concentration required to scavenge 50% of DPPH free radicals (SC_{50}; mg sample per mL methanol). All analyses were performed in triplicate.

Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP assay was used to assess the extract's antioxidant capability [29]. The FRAP reagent was prepared by mixing 25 mL of acetate buffer (300 mM, pH 3.6), 2.5 mL of 10 mM 2,4,6-tris(2-pyridyl)-s-triazine solution in 40 mM hydrochloric acid, and 2.5 mL of 20 mM iron (III) chloride hexahydrate solutions. Gallic acid was used as a standard ($r^2 = 0.999$). Results were given as µmol gallic acid equivalent per gram of the extract.

Cupric Ion Reducing Antioxidant Capacity (CUPRAC)

The cupric reducing antioxidant capacity of the extracts of *P. edulis* were determined using the method of Apak *et al.* (2004) [30]. First, 1 mL of Copper (II) chloride, neocuproin and ammonium acetate (1 M, pH=7) taken into a tube, the plant extract was added and the solutions were incubated for 30 minutes at room conditions. After 30 minutes, absorbance was measured at 450 nm. Gallic acid (Sigma Chemical Co) was used as a standard. Results of CUPRAC assay was presented as µmol gallic acid equivalent of one g extract.

Results and Discussion

Identification of Phenolic Compounds in the Extracts

The HPLC-DAD method was used to qualitatively and quantitatively analyse 15 phenolic standards in the extracts of *P. Edulis.* Ferulic acid and *p*-coumaric acid were determined as primary phenolic compounds in different

amounts in the three extracts. Protocatechuic acid, chlorogenic acid, caffeic acid, apigenin and isorhanmetin were also determined (Table 1). Gallic acid, vanillic acid, rutin, myricetin, quercetin and kaempferol were not detected in any of the plant extracts. The most abundant phenolic content was in the methanol extract, with the highest levels of ferulic acid (Table 1) and *p*-coumaric acid (Figure 1) than the other extracts. Methanol extract was the highest phenolic content among chloroform, ethyl acetate extracts.

Ferulic acid and *p*-coumaric acid, the major phenolic compounds in chloroform, ethyl acetate and methanol extracts of *P. edulis*, were reported as an important component of phenolic compounds with antioxidant properties and the ability to eliminate reactive oxygen [16; 31-33]. Six phenolic compounds such as isoorientin, isovitexin, orientin, vitexin, coumaric acid, rutin and quercetin have previously been determined in ethanolic extracts of *P. edulis* and *P. vitifolia* [13]. In another study, Lourith *et al.* [31] extracted *P. edulis* seeds with 40% methanol: water and fractionated with n-hexane and ethyl acetate. Kojic acid, gallic acid, chlorogenic acid, caffeic acid, ferulic acid, rosmarinic acid and quercetin were used as phenolic standards. Chlorogenic acid, caffeic acid, ferulic acid, and rosmarinic acid were identified in the ethyl acetate fraction. Rotta *et al.* [33] studied phenolic compounds and antioxidant potential of *P. edulis, P. alata* and *P. ligularis* fruits. 4-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, *p*-coumaric acid, ferulic acid, rutin, quercetin and trans-cinnamic acid were found in *P. edulis* fruit. The results were similar to the HPLC-DAD analysis in this work (Table 1). Another study determined phenolic profile of ethyl acetate extracts of fresh fruits of *P. cincinnata* and *P. edulis.* Gallic acid, catechin, epicatechin, epicatechin gallate, epigallocatechin-2,3,4-13C3 gallate, myricetin, rutin, quercetin, resveratrol, caffeic acid, chlorogenic acid, *p*-coumaric acid, and syringic acid were observed in different amounts in the two *Passiflora* species [34].

Table 1. Phenolic composition of *Passiflora edulis* extracts

Retention Time (Minute)	Standards	Extracts: Chloroform (mg std/g extract)	Ethyl acetate (mg std/g extract)	Methanol (mg std/g extract)
6.97	Protocatechuic acid	3.24	11.83	20.88
7.83	Chlorogenic acid	0.21	0.48	1.32
9.83	Caffeic acid	1.32	17.11	49.73
18.54	*p*-coumaric acid	45.87	86.30	116.88
19.20	Ferulic acid	99.14	132.79	175.12
28.51	Apigenin	24.61	34.77	85.60
29.72	Isorhamnetin	2.73	7.07	32.59

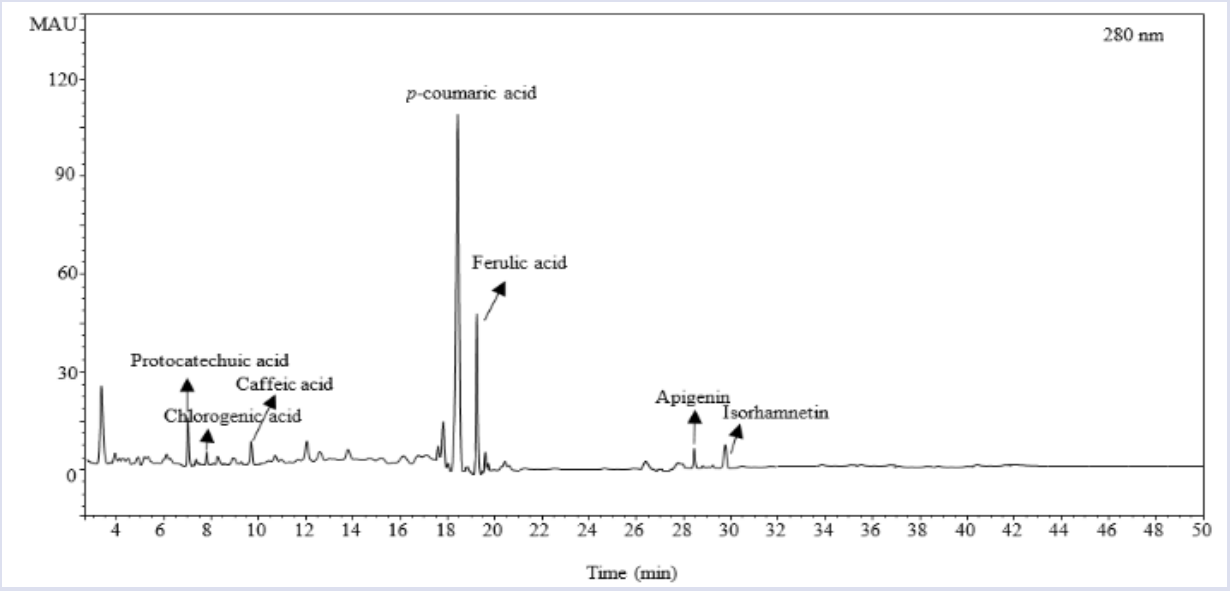

Figure 1. HPLC-DAD chromatogram of methanol extract of *Passiflora edulis*

Total phenolic (TPC) and Total flavonoid content (TFC)

TPC in the extracts of *P. edulis* were determined spectroscopically. According to experimental results, the highest TPF was obtained in the methanol extract of *P.edulis* as 825.09 ± 1.86 and the lowest was obtained in the chloroform extract as 142.57 ± 2.38 mg GAE/g (Table 2).

Total flavonoid content measured with aluminum chloride reagent and quercetin was used as a standard. TFC were in the extracts of *P. edulis* are presented in Table 2. According to the results of study, the highest TFC was obtained in the methanol extract as 502.78 ± 0.90 and the lowest was obtained in the chloroform extract as 106.76 ± 1.22mg QE/g.

Table 2. Total phenolic and flavonoid contents of the extracts from *Passiflora edulis**

	Chloroform	Ethyl acetate	Methanol
TPC (mgGAE/g)	142.57 ± 2.38	382.74 ± 0.95	825.09 ± 1.86
TFC (mgQE/g)	106.76 ± 1.22	377.78 ± 0.52	502.78 ± 0.90

*: Results were given as x̄ ± standard deviation

Lourith *et al.* [31] investigated total phenolic contents of ethyl acetate and water fractions of *P. edulis* seeds. The total phenolic contents were 58.3 g GAE/100 g for ethyl acetate fraction and 2.7 g GAE/100 g for water fraction, respectively. Colomeu *et al.*) [35] investigated methanol, ethanol and aqueous extracts of four *Passiflora* spp. They reported that TPC in those plants ranges from 0.023 to 0.228 mg GAE/g. Santos *et al.* [34] have used two Brazilian passion fruit species: *P. cincinnata* and *P. edulis.* Gallic acid used as the standard. The results of their study showed that the highest TPC was calculated *P. edulis* (476.1 mg GAE/kg) followed by that from *P. cincinnata* (365 mg GAE/kg). Another study reported the total phenolic and total flavonoid contents of ethanol extracts of *P. vitifolia* and *P. edulis* seeds. Results of that study showed the ethanol extract of *P. edulis* seeds were rich in total phenolic and total flavonoids [13].

Antioxidant Activity

In order to determine antioxidant activity, three extracts were analysed using radical scavenging (DPPH), reducing power (FRAP, CUPRAC) assays. Results of antioxidant assay were presented in Table 3.

Table 3. Antioxidant activities of the extracts from *Passiflora edulis**

Extracts	CUPRAC µmolGA/g	FRAP µmolGA/g	DPPH, SC50 mg/mL
Methanol	3.728 ± 0.150	0.511 ± 0.012	0.028 ± 0.001
Ethyl acetate	0.783 ± 0.051	0.358 ± 0.012	0.036 ± 0.002
Chloroform	0.161 ± 0.028	0.333 ± 0.018	0.345 ± 0.017
Gallic acid	-	-	0.004 ± 0.000
Quercetin	-	-	0.003 ± 0.000

*: Results were given as x̄ ± standard deviation

Lourith *et al.* [31] investigated antioxidant activity of *P. edulis* seed obtained from the residue after fruit juice production. Seeds of *P. edulis* was extracted with methanolic water and fractionated with n-hexane and ethyl acetate. Also, DPPH, FRAP and ABTS antioxidant tests were used. The ethyl acetate fraction showed the most potent antioxidant activity than other fractions. Additionally, the antioxidant activity of ethyl acetate extracts of *P. edulis* and *P. cincinnata* fruits was confirmed by Santos *et al*. [34] using DPPH, ABTS and FRAP methods. According to the results of their study, the fruits of *P. edulis* extract was higher than that of the *P. cincinnata*. Another study [32] have investigated antioxidant activity of aqueous, ethanol and methanol/acetone extracts of *P. edulis* fruits by using an ABTS, DPPH and FRAP methods. As a result of the study, the aqueous and ethanol extracts showed higher antioxidant activity when compared to methanol/acetone extract.

Conclusions

In this study, chloroform, ethyl acetate and methanol extracts of *Passiflora edulis* were investigated for the phenolic composition and antioxidant activities. Methanol extract of *P. edulis* was found to be the richest in total phenolic content and total flavonoid content. These findings were also supported by the results of HPLC-DAD analysis. For the phytochemical analysis, the methanol extract had the highest phenolic content among the chloroform, ethyl acetate extracts. Similarly, DPPH, FRAP and CUPRAC antioxidant tests showed that methanol extract had the highest antioxidant activity. In accordance with the results of the HPLC-DAD analysis, the extracts contain significant amounts of *p*-coumaric acid and ferulic acid, which are known to have potential biological activity. This study provided an important contribution to the existing knowledge on secondary metabolites of P. edulis. In conclusion, the fact that the plant has high antioxidant activity and is a good source of phenolic compounds may be encouraging in terms of expanding the biological properties of the plant. These studies may enable the plant to be used as a therapeutic agent in the future.

Conflict of interests

The author declare that she has no conflicts of interest.

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