A research on the detection of some phytochemical properties in the fruits of passiflora species

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Abstract. Passiflora belongs to the Passifloraceae family and is native to South Africa. Thanks to its health benefits, it is now commonly grown in tropical and subtropical regions. This fruit gathers attention, especially for its rich nutritional content, aroma, and taste. Passiflora has gained popularity in the Mediterranean region of Turkey, particularly in recent years. It stands out for its ease of maintenance, yielding twice a year, and high economic returns. Additionally, passiflora is used as an ornamental plant in landscaping arrangements by means of its showy flowers and is often referred to as the "passionflower" or "clock flower". In this study, the fruits of P. edulis and P. caerulea species were examined for their phytochemical properties, such as DPPH, total phenol, sugar, and organic acid. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and total phenol were analyzed using a spectrophotometric method, while sugar and organic acid were analyzed using HPLC.

1 Introduction

Passion fruit is a perennial, woody, and vining plant that belongs to the Passifloraceae family and is planted in tropical and subtropical countries. There are around 500 species in the world, but only about 60 of them can be consumed [1,2,3]. Passion flower is utilized in fresh and industrial use, as a decorative plant, and its many parts have medicinal characteristics over the world. The majority of these species are found in their natural habitats from the United States to Argentina, as well as in Australia and China on the Asian continent. Furthermore, Passiflora is a plant species native to South America, which includes Brazil, Colombia, Ecuador, Bolivia, and Paraguay. Brazil is considered its homeland because 89 passion flower species are indigenous to the country [4]. As a result, numerous passionflower species are grown commercially in South American indoor gardens. Brazil and Colombia, in particular, are among the countries that have traditionally grown passion fruit species [5]. In the world, commercially grown fruit species include sweet (*P. alata*), yellow-colored *(P. edulis f. flavicarpa*), and purple-colored (*P. edulis*). Yellow passion fruit orchards account for nearly 95% of commercial orchards [6, 7]. The edible section of passion fruit is high in vitamins A and C. It is also high in minerals including iron, potassium, sodium, magnesium, sulfur, and chloride. Passion fruit is utilized in a variety of processed products, including syrup, juice, and jam, in addition to fresh consumption. Furthermore, its fruits are extremely helpful to human health due to its high concentrations of fiber, vitamins, minerals, antioxidants, and various phytochemical components, as well as their cytotoxic, antioxidant, antihypertensive, antibacterial, and gastroprotective properties [8]. Fruits of Passiflora are high in organic acids, total phenol, antioxidants, and sugar compounds. A great deal of research has been conducted on this issue. [9] investigated the concentration of sucrose in mature fruits of *P. edulis*, *P. maliformis*, P. quadrangularis, *P. edulis* (yellow), *P. edulis*, flavicarpa, *P. edulis* (pink) species, and immature fruits of *P. edulis* (Pink) species. The highest sucrose content was determined as 45.51 ± 0.04 g kg^{-1} (fresh weight) in the immature fruits of the *P. edulis* (Pink) species. In the same study, the highest ascorbic acid was 0.32 ± 0.72 (g kg^{-1} FW) and the highest total phenolic content (TPC) was 362.00 ± 4.68 mg GAE L^{-1} FW in ripe fruits of *P. edulis* (purple) and the total antioxidant activity was (TAA) was detected as 1685.00 ± 9.82 mol Trolox L^{-1} FW in the mature fruits of P. maliformis species. In a study conducted by [10] on the determination of the phytochemical composition and antioxidant activity of Passiflora spp. varieties including Criollo PICH1, Criollo POR, Gulupa, Sweet passion fruit, P10, INIAP 2009; the highest total sugar content was detected as 52.47 ± 0.36 100 g^{-1} in Sweet Passion Fruit variety. On the other hand, the P10 variety had the greatest citric acid concentration of 31.77 ± 0.25 and 5.19 ± 0.04. In a study conducted to compare the impacts of high-pressure processing, pasteurization, and short-term high-temperature physicochemical qualities, nutritional quality, fragrance profile, and sensory properties of passiflora fruit puree; [11] determined the highest rates of fructose, glucose, and sucrose as 112.36 ± 2.93 g/L, 121.16 ± 2.69 g/L, 324.95 ± 5.47 g/L, respectively. They found the highest rates to be 0.59 ± 0.01 mg/mL oxalic

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acid, 2.84 ± 0.17 mg/mL malic acid, 2.31 ± 0.21 mg/mL lactic acid, 0.49 ± 0.15 mg/mL v, 29.78 ± 2.43 mg/mL citric acid, and 0.08 ± 0.01 mg/mL quinic acid According to the research, Passiflora fruit offers numerous benefits in terms of phytochemicals and human health. As a result, this study was conducted to assess the rates of change in sugar compositions, total phenol dpph (antioxidant) levels, and organic acid compositions in mature passion fruit or passiflora fruits under Adana climate.

2 Material and method

2.1 Plant material

Passiflora spp is grown in the experimental field of the Research and Application Centre at Cukurova University in Adana-Turkey during the 2019 growing season. Fruits were harvested from 4-year-old trees when they reached commercial maturity. Three replicates were taken from 3 different plants. MC1, MC2 and MC3 samples grown with a training system of 1.5m per row and 3 m between rows were taken. MC1: *Passiflora edulis*, harvesting time: the last week of September MC2: *Passiflora caerulea*, harvesting time: the last week of September. MC3: *Passiflora edulis*, harvesting time: the last week of October.

2.2 Biochemical parameters

2.2.1 Determination of sugar content

Glucose, fructose, xylose, sucrose, and total sugar content in the juice obtained from the harvested passifloras were determined according to the method developed by [12]. Before analysis, frozen juice samples were thawed at 25 ºC. A volume of 1 mL of juice was added to 4 mL of ultrapure water (Millipore Corp., Bedford, MA, USA). The reaction mixture was placed in an ultrasonic bath and sonicated at 80 ºC for 15 min and then centrifuged at 5500 rpm for 15 min and it was filtered before HPLC analysis (Whatman nylon syringe filters, 0.45 µm, 13 mm, diameter). The high-performance liquid chromatographic apparatus (Shimadzu LC 20A VP, Kyoto, Japan) consisted of an in-line degasser, pump, and controller coupled to a Refractive index detector (Shimadzu RID 20A VP, Kyoto, Japan) equipped with an automatic injector (20 µL injection volume) interfaced to a PC running Class VP chromatography manager software (Shimadzu, Kyoto, Japan). Separations were performed on a 300 mm x 7.8 mm i.d., 5 µm, reverse-phase Ultrasphere Coregel-87 C analytical column (Transgenomic) operating at 70 ºC with a flow rate of 0.6 mL min^{-1}. Elution was isocratic with ultrapure water. Individual sugars were calculated based on their standards and expressed in % of fresh weight (FW).

2.2.2 Determination of organic acids

Organic acids in passiflora fruit juice were determined by the HPLC analysis by [13] The malic, citric, succinic, fumaric, and L-ascorbic acid contents in passiflora juice samples were determined. For organic acids extraction, 1 mL of the sample, and 4 mL of 3% metaphosphoric acid were mixed. The mixture was placed in the ultrasonic water bath at 80 ºC for 15 min and it was sonicated and centrifuged at 5500 rpm for 15 min. Afterward, the mixture was filtered (Whatman nylon syringe filters, 0.45 µm, 13 mm, diameter) prior to HPLC analysis. The extract of organic acids was analyzed using a high-performance liquid chromatographic apparatus HPLC (Shimadzu LC 20A VP, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD 20A VP, Kyoto, Japan) and we used an 87 H column (5 µm, 300 mm x 7.8 mm (I.D.), Transgenomic). As for the operating conditions column temperature, was set at 40 ºC; injection volume, 20 µL; detection wavelength, 210 nm; flow rate 0.8 mL/min. and 0.05 mM sulphuric acid was used as the solvent. Identification of organic acids and determination of peaks is based on the retention times of peaks and comparison of spectral data according to standards. The identified acids were evaluated according to the relevant standard calibration curves. Results are expressed as mg 100 g^{-1}.

2.2.3 Determination of total antioxidant capacity

The radical scavenging activity of DPPH (2,2- Diphenyl-1-picrylhydrazyl) was done as described by [14] with slight modifications. Briefly, 0.06 µM of ethanolic DPPH was freshly prepared. Then, 1950 µL of DPPH was added to 50 µL of passiflora juice sample. The mixture was shaken for 1 min and kept in the dark for 30 min at room temperature. Absorbance was measured against the blank reagent at 515 nm. Radical scavenging activity %DPPH inhibition was calculated using the following equation: %Inhibition = 100x [(Abs blank (t = 30)) - (Abs sample)]/[(Abs blank (t = 30))] (1) where Abssample was the absorbance of the reaction in presence of sample (sample dilution + DPPH solution), Absblank was the absorbance of the blank for each sample dilution (sample dilution + DPPH solvent), and Abscontrol was the absorbance of control reaction (sample solvent + DPPH solution).

2.2.4 Determination of total phenol contents

The total phenolic content was determined by the Folin-Ciocalteau method [15] with some modifications. The analysis was done with a UV/VIS spectrophotometer (Thermo Fisher Scientific, FI-01620 Vantaa, Finland). Briefly, 9 mL of 80% methanol was added to 1 mL of the juice sample. The mixture was centrifuged at 5500 rpm for 10 min. A volume of 50 µL of supernatant was added to 250 µL of Folin- Ciocalteu reagent. Afterward, 750 µL 20% (w/v) Na_2CO_3 were supplemented, and it was incubated 2 h at room temperature. Then, the absorbance was measured at 760 nm against a blank. Quantifications were calculated through a calibration curve daily prepared with known concentrations of gallic acid (GA) standards, and results are expressed as mg GAE 100 g^{-1} fresh weight (FW) of passiflora.

2.3 Statistical analysis

The experiment was performed with a completely randomised experimental block design in three replications for three different samples (MC1, MC2 ,and MC3) in each replicate. Numerical calculations were performed with Microsoft® Office Excel for Mac®, version 11.1, 2004 (Microsoft® Corporation, Redmond, WA). The data were analysed with the statistical program JMP version 5.0.1 (SAS Institute Inc., Cary, NC, USA). ANOVA was performed to determine the effects of the samples on certain chemical parameters. A least significant difference test (LSD) was performed to examine the differences among samples and $p \leq 0.05$ was considered statistically significant [16].

3 Results and Discussion

Tables 1, 2, and 3 show the sugar compositions, total phenol dpph (antioxidant) concentrations, and change rates of organic acid compositions in passion fruit (passiflora) fruits grown under Adana climate. There was no significant difference between samples in terms of Dpph. On the other hand, in terms of total phenol contents of the samples, the difference between samples was found to be statistically significant. As for the total phenol content, highest value was obtained in MC3.

Table 1. Dpph, total phenol contents of Passiflora fruits.

Samples	Total Phenol (mg/100g GAE)	Dpph (%)
MC1	0.32±0.01 b	32.253± 2,59 a
MC2	0.33 ±0.03 b	32.302±0,30 a
MC3	0.41 ±0.05 a	35.982± 7.03 a
LSD0,05	0.057	13.782

[17] conducted a study to investigate the effect of extraction solvents on the total phenolic content and antioxidant activity of passiflora edulis extract. When they used ethanol extraction, they determined the total phenol content in fruit seeds as 9.249±0.04 mg GAE/g and in fruit juice as 0.0139±0.02 mg GAE/g. In our study, we obtained a variety of results regarding literature data. These variances are expected to vary based on the ripening seasons and growth conditions of the fruits.

Table 2. Sucrose, glucose, xylose and fructose contents of Passiflora fruits (mg/100g).

Samples	Sucrose	Glucose	Xylose	Fructose
MC1	3.10±0.08 a	3.48±0.03 b	0.04±0.01 b	3.25± 0.01 b
MC2	3.12±0.03 a	3.51± 0.05 b	0.05±0.00 ab	3.22±0.03 b
MC3	1.91±0.07 b	5.84 ±0.35 a	0.07±0.00 a	5.49±0.24 a
LSD0,05	0.221	0.658	0.018	0.460

Table 2 shows the sucrose, glucose, xylose and fructose contents (mg/100g) of Passiflora fruits. The difference between sugar content was found to be statistically significant. The highest sucrose content in fruit samples was 3.10 ± 0.08 mg/100g, while the lowest sucrose content was 1.91±0.07 mg/100g MC3. On the other hand, the highest glucose content in the samples was determined in MC3 with 5.84 ± 0.35 mg/100g and the lowest glucose content was determined in MC1 with 3.48±0.03 mg/100g. When the xylose level of the samples was tested, the highest xylose content was found to be 0.070.00 mg/100g in MC3 sample. In addition, the lowest xylose content in the samples was determined as 0.04±0.01 mg/100g. When the samples were examined in terms of fructose, the highest fructose content was found in MC3 as 5.49±0.24 mg/100g. The lowest fructose content was measured in MC2 with 3.22 ± 0.03. [18] employed two separate spectroscopic approaches, NIR and MIR, to detect specific sugars, organic acids, and carotenoids in Passiflora fruit. According to this study, glucose in fruit samples was determined as 23.64–217.38, fructose as 28.37–240.03, sucrose as 40.43–396.22, and total sugar as 92.44–762.89 mg g^{-1} DW. [19] stated that the maximum fructose content in fresh fruit juice was 0.59 g/100g in a study they did to investigate the chemical composition of raw fruit, peel, seed, and seed of Passiflora edulis. They reported that total phenol in fruit samples acquired from organic gardens was 64.48 ± 1.51mg GAE 100 g^{-1}, whereas total phenol in fruit samples collected from conventional gardens was 54.47 ± 0.74 mg GAE 100 g^{-1}. In the same study, researchers stated that while the DPPH values in organic samples were found to be 4.88 ± 0.13 (mg mL^{-1}), they determined the DPPH values in fruit samples taken from traditional gardens to be 6.87 ± 0.32 (mg mL^{-1}). In our study, we obtained a variety of results regarding literature data. These variances are expected to vary based on the ripening seasons and growth conditions of the fruits.

The concentrations of L - ascorbic acid, citric acid, malic acid, succinic acid, and fumaric acid in Passiflora fruits are shown in percentages in Table 3. In terms of organic acid contents of the samples, the difference between samples was found to be statistically significant. When the organic acid contents of the samples were tested, the results were discovered as follows. The highest L - ascorbic acid content was detected in MC2 as 5.96 ± 0.431%, and the lowest L - ascorbic acid content was found in MC1 as 4.83 ± 0.339%. The highest citric acid was found in MC2 as 799.59 ± 8.11%, and the lowest citric acid was detected in MC3 as 656.84 ± 22.82%. The highest malic acid was found in MC3 as 74.277± 2.67%, and the lowest malic acid was detected in in MC1 as 43.113± 2.23%. The highest succinic acid is was detected in MC3 at 487.04 ± 18.37 % while the lowest succinic acid was detected in MC1 at 289.36±28.82 %. The highest fumaric acid concentration was detected in MC3 at 3.13± 0.05%, and the lowest was found in MC1 at 2.85±0.1%. In a study conducted by [20], they examined *Passiflora glandulosa* fruit pulp extract and found its ascorbic acid content to be 57.76 mg/100g. In another study on *passiflora cincinnata* and *passiflora edulis* conducted by [21] the citric acid content in the *Passiflora cincinnata* was found to be 6.85 ± 0.25 g 100 g^{-1}, while the citric acid content in the *Passiflora edulis* was determined to be 3.87 ± 0.80 g 100 g^{-1}. In addition, while the malic acid content in *Passiflora cincinnata* was found to be 1.66 ± 0.05 g per 100 g^{-1}, the malic acid

content in *Passiflora edulis* was found to be 1.60 ± 0.29 g per 100 g^{-1}. In addition, the succinic acid content in *Passiflora edulis* was detected as 0.08 ± 0.03 g per 100 g^{-1}, and the succinic acid content in *Passiflora cincinnata* was found as 0.17 ± 0.05 g per 100 $g^{-1.}$ In our study, we obtained a variety of results regarding literature data. These variances are expected to vary based on the ripening seasons and growth conditions of the fruits.

Table 3. L - ascorbic acid, citric acid, malic acid, succinic acid and fumaric acid contents of Passiflora fruits (%).

Samples	L-ascorbic acid	citric acid	malic acid	succinic acid	fumaric acid
MC1	4.83 ± 0.339 b	712.89 ± 48,18 b	43.113 ± 2,228 c	289.36 ± 28.82 c	2.85 ± 0.1 b
MC2	5.96 ± 0.431 a	799.59 ± 8,11 a	48.9231 ± 1,076 b	346.43 ± 12.349 b	2.92 ± 0.05 b
MC3	5.60 ± 0.147 a	656.84 ± 22,82 b	74.277 ± 2,667 a	487.04 ± 18.373 a	3.13 ± 0.05a
LSD0,05	0.655	62.20	4,196	41.92	0.140

4 Conclusion

In this study, significant differences in biochemical content were obtained in passiflora fruits. As a consequence, in terms of total phenol content, MC3 had the highest total phenol content. In terms of total antioxidant activity DPPH, MC3 has the highest DPPH value. In passiflora fruits, the highest sucrose concentration was identified in MC2 fruits, while the highest glucose, fructose, and xylose content was found in MC3 fruits. While the highest L - ascorbic acid and citric acid contents were found in MC2, the highest malic acid, succinic acid and fumaric acid contents were detected in MC3. We believe that this study will shed light on more detailed studies in the future.

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