АРОМАТИЧЕСКИЕ И ЛЕКАРСТВЕННЫЕ РАСТЕНИЯ

УДК 582.001.4
DOI 10.36305/2712-7788-2023-1-166-25-32

PHYTOCHEMICAL ANALYSIS OF SOME SPECIES BELONGING TO THE *PASSIFLORACEAE* FAMILY

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The article is dealing with biochemical analysis of ethanol extracts of leaves and fruits from *P. carnata* L., *P. edulis* Sims., *P. caerulea* L., *P. ligularis* Juss. and *P. foetida* L. species belonging to the *Passiflora* L. genus. Using the methods DPPH, ABTS, oxidation of ß-carotene/linoleic acid, CUPRAC and FRAB, a comparative study of the total content of phenolic substances, flavonoids, tannins and antioxidant activity was carried out. According to the results, the ROS scavenging activity of the extracts changed between 1.36 ± 0.05 and 2.43± 0.25 IC_{50} mg/ml (the DPPH method) and between 0.25±0.012 and 2.48±0.016 IC_{50} mg/ml (the ABTS method). In the assay performed with ß-carotene/linoleic acid, the lowest value (45±1.21%) of the studied parameter was found for the fruit extracts of *P. caerulea* L. The FRAB analysis showed the highest antioxidant activity (1.61±0.08 mg TE/g) in leaf extracts of *P. incarnata* L. The highest inhibition level was found in leaf extracts of *P. incarnata* L. (83±0.53%) and *P. caerulea* L. (81±0.8). The highest cupric ion (II) reducing antioxidant capacity was detected in *P. caerulea* L. leaf (7.29 ± 0.33 mgTE/ g), and the lowest one was in *P. edulis* Sims. (2.47 ± 0.07 mgTE / g) fruit extracts.

The results of our phytochemical analysis proved that the extracts of local plant species, which have long been used in the treatment of various diseases, are rich in bioactive compounds of medicinal importance.

Keywords: *antioxidant; extraction; phytopreparation; introduction; introducent*.

The *Passiflora* L. genus belonging to the *Passifloraceae* family has about 600 species (Ayres et al. 2015; Wosch et al. 2017). It is estimated that there are 83 species in Brazil and more than 140 species have been formed (Gomes et al., 2017). Of them, 60 species have been confirmed to have edible fruits (Pertuzatti et al., 2015). The rich and colorful vegetation of Azerbaijan provides ample opportunities for the development of pharmacognostic direction in the search for biologically active substances, determination of areals of medicinal plants and raw material reserves, as well as the production of phytopreparations. In general, some species of the genus *Passiflora* L. have been officially registered in Pharmacopoeias (EP 2013, TF 2017) of many countries and various institutions (EMA 2013, ESKOP 2003, FFD 2017), including Homeopathic Pharmacopoeias for the treatment of central nervous system diseases such as insomnia and anxiety due to their sedative and anxiolytic effects.

In recent years, the importance of medicinal plants in terms of the psychological and physical health of people has increased. Due to the positive effects of treatment with medicinal plants on human health, scientists have accepted that it is an alternative method in the treatment of diseases of the nervous system. Some species of the *Passiflora* L. genus have been used as a source of therapeutic drugs for many years. Their use as a medicinal plant in traditional medicine dates back to the past (Shahid et al., 2013). According to the literature,

most *passiflora* species grow in South America, and in the form of tea have been used in folk medicine by the American Indians. Today, these plants are an integral part of phytopharmaceutical products worldwide. *Passiflora* L. species, rich in biologically active substances were studied and these substances were isolated. These studies were also performed on phenols and flavanoids (Pereira et al., 2004). "*Passicol*", one of the chemical compounds in *Passiflora* L. flowers, was reported to have antimicrobial activity (Nicolls et al., 1973; Mohanasundari et al., 2007). Organic substances in plants, unlike synthetic drugs, are distinguished by their closeness to the human organism. Therefore, their bioassimilation is higher (Yusif Karimov et al., 2010).

In addition to the sedative effect, the *P. incarnata* L. species has a strong antioxidant effect on the human organism (Kim et. all., 2017). Phytochemical analysis of biologically active substances in leaf and fruit ethanol extracts of *P. incarnata* L, *P. edulis* Sims., and *P. caerulea* L., introduced for the first time in Azerbaijan was performed in the experimental field of the Institute of Dendrology of ANAS for the comparative phytochemical study.

The introduction is a complex biological process. When carrying out this process, the tolerance of the introducent, its adaptation to climatic conditions (temperature, air, and soil moisture, light), genetic characteristics, as well as its geographical origin must be known. It is necessary to study the biological properties of the plant, which are formed as a result of interaction with the environment. Since this process is applied to medicinal plants, one of the most important factors is to control their chemical compositions, preventing them from significant changes due to the new environment. The introduction of a plant can be planned only after studying all the factors, including thermal, bioecological, geographical, and chemical complex factors, and finding the integral and functional relations between them.

P. edulis Sims., *P. incarnata* L., *P. caerulea* L, *P. ligularis* Juss. *P. foetida* L. species are plants with economic and medicinal value, producing fruits with a large number of seeds. In recent years, foods rich in antioxidants have been identified and their use in human nutrition has become the focus of attention (Halliwell, 2001). Fruits, which play an important role in a healthy diet, are also said to have high antioxidant activity.

Several studies showed that dried fruits are an excellent source of antioxidants (Vinson et al., 2005). The role of antioxidants in the prevention of various degenerative and age-related diseases caused by oxidative stress is considered to be even more important after clinical and epidemiological studies have revealed them. Various epidemiological studies have shown that consuming lots of fruits and vegetables reduces the risk of cancer and cardiovascular diseases (Can et al., 2005).

The most studied biological activity of the passion fruit is antioxidant action that is attributed to polyphenols, particularly, flavonoids (Heim, Tagliaferro, and Bobilya, 2002).

Currently, the demand for tropical plants is growing rapidly. Cultivation of passiflora species, tropical plants introduced in Azerbaijan, provides a basis for research on the richness of biologically active substances in them, the identification of new types of raw materials for their production, as well as the study of the impact properties of these substances. Many studies have focused on the discovery of natural sources and clinically useful antimicrobial drugs and functional foods for pharmaceutical and nutraceutical use (Davies, 1994; Sajid et al., 2012).

The purpose of our research was to determine the total amount of biologically active substances in *P. edulis* Sims., *P. incarnata* L., *P. caerulea* L, *P. ligularis* Juss., *P. foetida* L. genus, introduced for the first time in Azerbaijan using various phytochemical analysis methods under the local conditions.

Materials and methods

Chemicals: DPPH, ABTS, BHA, ß-carotene, Trolox, FCR, Sodium carbonate, gallic acid, and quercetin were obtained from Sigma-Aldrich (USA).

Research objects: The aim of our research was to determine the content of biologically active substances and antioxidant activity of extracts from fruits and leaves of *P. edulis*, *P. incarnata*, *P. caerulea*, *P. ligularis*, *P. foetida* introduced for the first time in Azerbaijan. *P. edulis* Sims., *P. incarnata* L., *P. caerulea* L, *P. ligularis* Juss., *P. foetida* L. are the promising species of the *Passiflora* L. genus, introduced in the experimental field of the Institute of Dendrology of the Azerbaijan National Academy of Sciences in 2018, were chosen as the research objects. The identification and botanical naming of the specimens were based on the scheme of Ulmer and MacDougal (Ulmer and MacDougal, 2004). The introduction of all four species has been successfully completed. The above-ground parts of the plants (leaves and ripe fruits) were collected and dried separately under a tent equipped with special racks to prevent their exposure to sunlight and moisture.

Preparation of extracts: To study the antioxidant activity, solutions of 1 mg/ml of ethanolic extracts from leaves and fruits of *P. edulis* Sims., *P. incarnata* L., *P. caerulea* L., *P. foetida* L., *P. ligularis* Juss. were prepared and analyzed. The extracts were prepared under laboratory conditions using the Maceration method. After the drying process, raw materials were cleaned of the defective parts to provide a condition meeting the requirements of normative and technical documents (Yusif Karimov et al., 2010).

The research was conducted in the Natural Products and Biotechnology Laboratory of the Mughla Sitki Kochman University of the Republic of Turkey.

The dried plant samples were separated into small pieces using a blender and 20 grams of samples were collected in Erlenmeyer flasks, 100 ml of a solvent (methanol, water, or acetone) was added, shaken, and kept in a water bath at 48- 50°C for 6 hours. Then, it was poured through a filter paper (Whatman No. 2), into a volumetric flask, which was round and swollen at the bottom and cylindrical at the top, and the solvent was added again and kept for 6 hours. After filtration, the samples were placed in a Rotary Evaporator to completely remove the solvent. After the solvent had evaporated, the remaining extracts in the flask were poured into Petri dishes with water. The extract samples were kept in a lyophilizer at – 54˚C, for 8-10 hours to completely remove the water inside. Samples from the lyophilization process were scraped from the Petri dishes with a spatula and placed in glass jars with lids. The samples were stored at -20°C until used (Turan and Mammadov, 2018).

Determination of Antioxidant Activity

DPPH Free Radical Scavenging Activity assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity assay was carried out using the method of Turan and Mammadov (2018). 4ml of the DPPH solution was added to 1ml of BHA (for positive control) and extract solutions of different concentrations (1.0, 1.2, 1.4, 1.6 mg/mL) were prepared. As a negative control, 4ml of the DPPH solution was added to 1 ml of the solvent. It was measured at 517 nm after 30 minutes of incubation in the dark at room temperature. The percentage of antioxidant activity value (AA%) was found according to the following formula (1):

$$AA\% = [(A_c - A_s) / A_c] \times 100 \quad (1)$$

A_c; Absorbance of the control, A_s; Absorbance of the extract or standard

ABTS radical cation scavenging activity assay

The method of Re et al. (1999) was used for the ABTS (2,2-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay. 4.5 mL of the ABTS solution was added to 0.5 mL extract or standard solutions (BHA) prepared at five different concentrations (0.05-0.25 mg/mL). As a negative control, 4.5 mL of the ABTS solution was added to 0.5 mL of solvent. It was measured at 734 nm after incubation for 30 minutes at room temperature. The percentage of antioxidant activity (AA%) was found according to the following formula (2):

$$AA\% = [(Ac - As)/Ac] \times 100 \quad (2)$$

Ac; Absorbance of the control, As; Absorbance of the extract or standard

β-Carotene/Linoleic Acid Method

The method developed by Amarowicz et al. (2004) was used in the β-carotene/linoleic acid assay. 24 mL of the β-carotene solution was added to 1 mL of the extract or BHA (for positive control). First, the initial absorbance of the mixtures was measured at 470 nm. Afterward, it was incubated at 50 °C for 2 hours and measurements were made every half an hour. As a negative control, 24 mL of the β-carotene solution was added to 1 mL of the solvent. Total antioxidant activity was calculated using the following formula (3):

$$AA\% = 1-[(Ao(0)-Ac(0))/(Ao(d)-Ac(120)) +(As(0)-As(120)))] \times 100 \quad (3)$$

Ao (0); The absorbance of the extract at 0 min, Ac(0); The absorbance of the control of the extract at 0 min, Ao(120); The absorbance of the extract at 30, 60, 90, or 120 minutes, Ac(d); The absorbance of the control of the extract at 30, 60, 90, or 120 minutes, As(0); The absorbance of the positive control at 0 min, As(120); It represents the absorbance of the positive control at 120 minutes.

FRAP (Ferric ion reducing antioxidant power) method

The method developed by Benzie and Strain (1996) was used in the experiment. To determine the FRAP, 2 mL of the FRAP solution was added to the 1 mg/mL extract solution. It was then measured at 595 nm after keeping it at room temperature for 30 minutes. Trolox (0.05-0.2 mg/mL) was used as a standard. The calibration curve was constructed using the standard.

CUPRAC (Cupric ion reducing antioxidant capacity assay) method

The cupric ion reducing antioxidant capacity assay (CUPRAC) was performed according to the method described by Apak et al. (2004). To determine the CUPRAC, 3 mL of the CUPRAC solution was added to 0.5 mL of the extract solution (1 mg/mL) and then incubated for 30 minutes at room temperature and measured at 450 nm. Trolox (0.01-0.05 mg/mL) was used as a standard. The calibration curve was constructed using the standard.

Quantification Analysis of Extracts.

Quantification of Total Phenolic compounds

Quantification of total phenolic compounds was carried out according to the method developed by Singleton and Rossi (1965). To determine the content of phenolic compounds, 1 mL of the Folin-Ciocalteu reagent (FCR) was added to the 1 mg/mL extract solution. After adding 46 mL of distilled water, it was kept for 3 minutes. After 3 minutes, 3 mL of sodium carbonate was added and kept at room temperature for 2 hours. The measurement was made at 760 nm after 2 hours. Gallic acid (0.01-0.05 mg/mL) was used as a standard. The calibration curve was constructed using the standard.

Quantification of Total Flavonoids

Determination of total flavonoids was conducted according to the method of Aryal et al. (2019) with some modifications. To determine the amount of flavonoids, 0.2 mL of sodium acetate was added to the 1 mg/mL extract solution. Then, 1 mL of aluminum chloride and 5.6 mL of distilled water were added. It was measured at 415 nm after 30 minutes at room temperature. Quercetin (0.01-0.05 mg/mL) was used as a standard. The calibration graph was constructed using the standard.

Quantification of Total Tannins

The Bekir et al. (2013) method was used for determining the content of total tannins. 0.5 mL of the extract or 1.5 mL of the vanillin solution was added to the standard of different concentrations in test tubes in an ice-filled container. After the vanillin solution was added, it was incubated at room temperature for 15 minutes. After 15 minutes, its absorbance was measured at 500 nm. For the calibration curve, the same procedures were performed for catechin solutions at different concentrations (0.01 mg/mL, 0.02 mg/mL, 0.03 mg/mL, 0.04 mg/mL, 0.05 mg/ml)

Statistical Analysis

All experiments with each concentration were conducted in three replicates and the results were presented as the mean ± standard error. Statistical analyses were performed using Microsoft Office Excel software. The results were evaluated using an unpaired t-test and one-way analysis of variance ANOVA (Minitab). The differences were regarded as statistically significant at $p<0.05$.

Results and dıscussıon

The antioxidant activity of *Passiflora* L. extracts for DPPH, ABTS assays, the IG_{50} value, and the percentage value for ß-carotene/linoleic acid assays are shown in Table 1. The ROS scavenging activity of the extracts changed between 1.36 ± 0.05 and 2.43± 0.25 IC50 mg/ml (the DPPH method) and between 0.25±0.012 and 2.48±0.016 IC50 mg/ml (the ABTS method).

Table 1

Antioxidant activity of leaf and fruit ethanol extracts of species belonging to the *Passiflora* L. genus

Plants	DPPH (IC50, mg/mL)	ABTS (IC50, mg/mL)	β-Carotene/ Linoleic Acid (%)	FRAP (mg/mL)	CUPRAC (mg/mL)
Passiflora caerulea L. - L*	1.38±0.09	0.4±0.009	81±0.8	0.81±0.12	7.29±0.33
Passiflora caerulea L. - F.**	1.80±0.09	0.42±0.008	45±1.21	0.49±0.02	4.26±0.06
Passiflora edulis Sims. - L.*	2.08±0.06	0.33±0.008	71.05±3.65	1.50±0.10	6.06±0.20
Passiflora edulis L. - F.**	1.41±0.02	0.48±0.016	63±2.18	0.67±0.02	2.47±0.07
Passiflora incarnata L.- L*	1.44±0.03	0.3±0.005	83±0.53	1.61±0.08	2.83±0.15
Passiflora incarnata L. - F**	1.36±0.05	2.48±0.016	65±2.11	1.55±0.10	5.02±0.14
BHA	0.007±0.0002	0.0082±0.004	88±0.54	-	-

*Leaf ethanol extract, **Fruit ethanol extract.

The determination of the antioxidant activity of *Passiflora* L. extracts showed the highest free radical scavenging activity in the *P. incarnata* L. ethanol extract among all the studied extracts. In ß-caroteno/linoleic acid experiments, the highest inhibitory effect in Passiflora ethanol extracts was observed in leaf extracts of *P. incarnata* L. (83 ± 0.53%) and *P. caerulea* L. (81 ± 0.8).

The lowest inhibition level was observed in *P. caerulea* L. fruit extracts (45 ± 1.21%). Table 1 shows the ferric ion reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) of the extracts. FRAP of the extracts changed between 0.49±0.02 and 1.61± 0.08TE/g. In terms of FRAP, the highest antioxidant activity (1.61±0.08 mgTE/g) was detected in the leaf extract of *P. incarnata* L. Whereas, the lowest activity (0.49±0.02 mg TE/g) was observed in the fruit extract of *P. caerulea* L. Cupric (II) ion reducing antioxidant capacity of the extracts changed between 2.43±0.16 and 9.01±0.38 mgTE/g. The highest cupric ion (II) reducing antioxidant capacity was detected in the *P. caerulea* L. leaf extract (7.29 ± 0.33 mgTE/g), while the lowest one was found in the *P. edulis* Sims. (2.47 ± 0.07 mgTE/g) fruit extracts. Table 2 shows the total secondary metabolite amounts of *Passiflora* L. ethanol extracts. Thus, the total amount of phenolic compounds in Passiflora extracts varies between 0.96 ± 0.05 and 3.16 ± 0.18 mgGAE/g. The largest amount of phenolic compounds was found in the *P. caerulea* L. leaf extracts (3.16 ± 0.21 mgGAE/g), while the smallest one was observed in the ethanol extracts of *P. caerulea* L. fruit (0.96 ± 0.05 mg GAE/g). Total flavonoids changed between 0.65±0.07 and 12.52±1.85mgQE/g. The

highest total flavonoid amount was found in the 1 mg/Ml leaf extracts of *P. caerulea* L.. Total tannins changed between 0.22±0.13 and 4.33±0.16 mgGAE/g. The largest amount (4.33±0.16 mgGE/g) of tannins was found in the fruit extract of *P. caerulea* L. Whereas, the smallest amount (0.22±0.13 mg GAE/g) of tannins was detected in the fruit extract of *P. edulis* Sims.

Table 2

Total secondary metabolite content of leaf and fruit ethanol extracts of some species of the *Passiflora* L. genus

Plants	Total amount of phenolic compounds (mg GAE/g)	Total amount of Flavonoids (mg QE/g)	Total amount of Tannins (mg CE/g)
Passiflora caerulea L. - L*	3.16±0.21	12.52±1.85	0.75±0.15
Passiflora caerulea L. - F**	0.96±0.05	6.34±1.48	4.33±0.16
Passiflora edulis Sims. - L*	2.42±0.35	9.56±1.56	0.65±0.031
Passiflora edulis Sims. - F**	1.44±0.26	1.23±0.24	0.22±0.13
Passiflora incarnata L. - L*	1.32±0.12	8.57±0.59	3.2±0.07
Passiflora incarnata L. - F**	2.37±0.24	9.32±1.45	2.05±0.04

*Leaf ethanol extract, **Fruit ethanol extract.

Masteikova et al. (2008) compared the antioxidant activities of aqueous and 70% ethanol leaf extracts of *P. incarnata* L. and reported that ethanol extracts were more effective free radical binders than aqueous extracts.

The results of the analysis of other researchers differed from our results. Thus, in the passiflora leaf extracts: total phenolic compounds, total flavonoids, DPPH free radical scavenging activity, and ferric ion reducing antioxidant power amounted to 22.92 ± 0.18 mg GAE/g, 7.01 ± 0.10 mg CE/g, 2.77 ± 0.02 mg GAE/g, and 3.20 ± 0.04 mg GAE/g, respectively. In fruit extracts: total phenolic compounds, total flavonoids, DPPH free radical scavenging activity, and ferric ion reducing antioxidant power amounted to 6,53±1,02 mg GAE/g, 1.56±0.27 mg GE/g, 1.00±0.15 mg GAE/g, and 1.12±0.17 mgGAE/g, respectively (Yohanes et al., 2020).

Conclusions

The results show that *Passiflora* L. extracts are not only sedative but also have high antioxidant activity.

The extracts of passiflora species contain about 20 flavonoid compounds, and 9 of them were identified as isovitexin, vitexin, rutoside, hyperoside, luteolin, kaempferol, kempferitrin, orientin, and isoorient (Guseinov et al., 2010).

Some scientists suggested that the antioxidant properties are due to flavonoids of the genus *Passiflora* L. (Rudnicki et al., 2007; Masteikova et al., 2008).

The results of our phytochemical analysis proved that the extracts of these plant species, which have long been used in the treatment of various diseases, are rich in bioactive compounds of medicinal importance. The positive results obtained from the phytochemical analysis of *P. edulis* Sims., *P. incarnata* L., *P. caerulea* L, *P. ligularis* Juss., *P. foetida* L. species will make a significant contribution to the healthcare system and the development of the pharmaceutical industry of the world in the future. The introduction of valuable medicinal *Passiflora* L. species plants in the world will eliminate dependence on chemical preparations in terms of medicinal raw materials in the future and at the same time, it will stimulate the development of new plant-based medicine and food industry.

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Статья поступила в редакцию 07.07.2022 г

Бадалова В.Н., Мамедова З.А., Сулейманов Т.А., Атай М.О. Фитохимический анализ некоторых видов семейства *Passifloraceae* // Plant Biology and Horticulture: theory, innovation. 2023. № 1(166) P. 25-32.

Статья посвящена биохимическому анализу этанольных экстрактов листьев и плодов видов *P. incarnata* L., *P. edulis* Sims. и *P. caerulea* L., *P. ligularis* Juss. и *P. foetida* L., принадлежащих к роду *Passiflora* L. С помощью методов DPPH, ABTS, окисления ß-каротина/линолевой кислоты, CUPRAC и FRAB проведено сравнительное изучение суммарного содержания фенольных веществ, флавоноидов, танинов и антиоксидантной активности. Согласно результатам, активность экстрактов по удалению АФК изменялась от 1,36 ± 0,05 до 2,43 ± 0,25 IC50 мг/мл (метод DPPH) и от 0,25 ± 0,012 до 2,48 ± 0,016 IC50 мг/мл (метод ABTS). В пробе с ß-каротином/линолевой кислотой наименьшее значение (45±1,21%) изучаемого показателя было обнаружено для экстрактов плодов *P. caerulea* L. FRAB-анализ показал наибольшую антиоксидантную активность (1,61±0,08 мг ТЭ/г) в экстрактах листьев *P. incarnata* L. Наиболее высокий уровень ингибирования обнаружен у экстрактов листьев *P. incarnata* L. (83±0,53%) и *P. caerulea* L. (81±0,8). Наивысшая антиоксидантная способность по снижению содержания ионов меди (II) выявлена в листьях *P. caerulea* (7,29 ± 0,33 мг ТЕ/г), а наименьшая – в экстрактах плодов *P. edulis* Sims. (2,47 ± 0,07 мг ТЕ/г). Результаты проведённого нами фитохимического анализа доказали, что экстракты местных видов растений, издавна применяемых при лечении различных заболеваний, богаты биологически активными соединениями, имеющими лечебное значение.

Ключевые слова: *антиоксидант; экстракция; фитопрепарат; интродукция; интродуцент*.