

Flavonoids in *Passiflora incarnata* L. Dry Extract of Russian Origin

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ABSTRACT

Background: Flavonoids are one of the main classes of biologically active substances providing the pharmacotherapeutic effect of passionflower (*Passiflora incarnata* L.) preparations. In this article studies on the standardization of *Passiflora incarnata* L. dry extract (PDE) by flavonoids are presented. The aim of this work was to study the composition and content of flavonoids in PDE with the help of precise modern physicochemical methods.

Materials and methods: PDE was prepared from crude herbal drug – *P. incarnata* herb of Russian origin. Reverse phase HPLC-UV analysis was performed with Agilent 1100 liquid chromatograph. Chromatographic column was Atlantis C₁₈ (250 mm × 4.6 mm × 5 μm); analytical wavelength – 350 nm; mobile phase – 0.01% formic acid solution and methanol: acetonitrile (25:75); column temperature – 35°C; analysis time – 90 min; flow rate of the mobile phase – 0.8 ml/min in gradient elution mode. Commercially available samples of flavonoids were used for identification and quantitative determination.

Results: 20 compounds of flavonoid structure are presented in the PDE. 9 flavonoid compounds have been identified, they are: isovitexin, vitexin, rutoside, hyperoside, luteolin, kaempferol, kaempferitrin, orientin, and isoorientin. The content of vitexin is 0,867 ± 0,011%, the total flavonoids content in terms of vitexin is 3,762 ± 0,049%.

Conclusion: The obtained data will be used to create regulatory documentation for drugs based on PDE.

Key words: *Passiflora incarnata*, Dry extract, Standardization, Flavonoids.

INTRODUCTION

Passiflora incarnata L. or purple passionflower (Figure 1) – a perennial herbaceous tropical plant of the family Passionflower (*Passifloraceae* Juss. Ex Roussel). The *P. incarnata* homeland is tropical Brazil, Bermuda, as well as subtropical regions of North America.¹ *P. incarnata* is introduced in Kobuleti along the Black Sea coast of the Caucasus in Russia, where industrial plant areas are located.²

Currently, alternative treatment of patients with herbal drugs (including passionflower) with anxiety disorders remains extremely urgent. *P. incarnata* herb contains about 0.04% β-carboline alkaloids – harmine, harmaline, harmalol.³ Also *P. incarnata* herb includes various groups of biologically active substances (BAS): chlorophyll and pectin substances, saponins, vitamins,⁴ oxycoumarins;⁵ a complex of flavonoids (vitexin, quercetin, luteolin, apigenin), which has sedative activity; coumarins, quinones.⁶

P. incarnata herb contains a wide variety of flavonoid glycosides. The presence of vicenin-2, schaftoside, isoschaftoside, orientin, isoorientin-2"-O-β-glucopyranoside, isoorientin, isovitexin-2"-O-β-glucopyranoside, swertisin, isovitexin, vitexin were established by group of Italian researchers.⁷ A team of researchers from Austria and Germany identified flavone C-glycoside – isoscaparin-2"-O-glucoside in the *P. incarnata* herb.⁸ Also 2-O-Glucosyl-6-C-glucosylapigenin, 6-β-D-glucopyranosyl-8-β-D-ribopyranosyl apigenin were isolated from *P. incarnata* herb.^{9,10}

In the extract of *P. incarnata*, the following hydroxycinnamic acids are found: a derivative of caffeic acid, p-coumaric acid glycoside and chlorogenic acid. Flavonoids are present in the form of apigenin, kaempferol, quercetin glycosides.¹¹

The *P. incarnata* crude herbal drugs (CHD) are included in the European Pharmacopoeia,¹² the State Pharmacopoeias of Germany,¹³ France¹⁴ and the United Kingdom,¹⁵ the British herbal pharmacopoeia.¹⁶ In Russia there is pharmacopoeial monograph 42-2784-91 regulating the quality of the crushed herb of passionflower.¹⁷

PDE (Figure 2) in combination with extracts of other CHD is a part of domestic and foreign drugs "Stressoff Forte", "Stressoff" (JSC "Vifehtech", Russia), "Novopassit" (Czech Republic, Israel), used as sedatives;¹⁸ and as part of complex homeopathic remedies for the treatment of alcohol dependence.¹⁹ *Passiflora* extracts have a confirmed anxiolytic activity.²⁰ In an experiment in mice, it was found that, in addition to the neuroprotective effect, a decrease in the level of neuropathic pain is characteristic for passionflower.²¹ Base antinociceptive mechanisms are carried out due to the effect on opioid, GABA receptors. Sedative action is provided through GABA-ergic mechanisms, due to which a dose-dependent effect is realized.²¹ Flavonoids can possess antiepileptic properties.²²

Since flavonoids are one of the main classes of BAS providing the pharmacotherapeutic effect of *passiflora* preparations, it was necessary to determine their qualitative composition and content with the

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Figure 1: Purple passionflower (*Passiflora incarnata* L.).



Figure 2: *Passiflora incarnata* dry extract.

use of precise modern physicochemical methods, one of which is HPLC-UV (DAD).²³

The purpose of this work is to study the composition and content of flavonoids in *P. incarnata* herb dry extract.

MATERIAL AND METHODS

P. incarnata herb dry extract (70% ethanol, ratio of CHD and extragent is 1: 2) was investigated. The *P. incarnata* herb was harvested during the flowering phase – the beginning of fruiting in the Krasnodar Territory (Sochi, Dagomys settlement) in 2016. *P. incarnata* plants have been identified by Bokov Dmitry Olegovich, associate professor of Pharmaceutical Natural Sciences Department, voucher specimens were deposited at the Pharmaceutical Natural Sciences Department Herbarium.

Commercially available samples of individual substances: luteolin-7-O-glucoside (Sigma-Aldrich, CAS No. 5373-11-5, ≥98%), vitexin (Sigma-Aldrich, CAS No. 3681-93-4, ≥95%), isovitexin (Sigma-Aldrich, CAS No. 38953-85-4, ≥95%), rutin trihydrate (Sigma-Aldrich, CAS No. 250249-75-3, ≥94%), luteolin (Sigma-Aldrich, CAS No. 491-70 -3, ≥ 97%), hyperoside (Sigma-Aldrich, CAS No. 482-36-0, ≥97%), kaempferol (Sigma-Aldrich, CAS No. 520-18-3, ≥99.0) were used as standard samples (SS).

Investigations of the composition and content of individual flavonoids in PDE were carried out on an Agilent 1100 liquid chromatograph. The chromatograph is equipped with a two-solvent feed and degassing system, a diode array detector, a column thermostat, and an autosampler. Data collection, processing of chromatograms and absorption spectra were obtained with the help of Agilent ChemStation. Atlantis C₁₈ chromatographic column

(WATERS, USA) was used for separation of flavonoid compounds. It is reversed-phase HPLC (250 mm in length and 4.6 mm in internal diameter, with a particle size of 5 μm, filled with silica gel chemically bonded to octadecylsilane). The wavelength of the detection corresponded to the local maxima (peaks), it was selected after recording the absorption spectra at wavelengths of 190-700 nm. 350 nm was the analytical wavelength. A solvent system of 0.01% formic acid solution and methanol: acetonitrile (25:75) (for HPLC, PanReac AppliChem) was used as the mobile phase; column temperature was 35°C; analysis was lasting for 90 min. The flow rate of the mobile phase is 0.8 ml / min. The maximum pressure is 400 bar. The volume of the injected sample is 10 μl. The gradient elution scheme is shown in Table 1.

The PDE preparation for the HPLC analysis was carried out in accordance with the methodology presented below.

Method of PDE preparation for the flavonoid analysis

To determine the flavonoid content in PDE, the 50 mg (accurately weighed quantity) of the extract was dissolved in 2 ml of the solvent (40% ethanol), placed in centrifuge tubes and centrifuged for 15 minutes at a rate of 4,500 rpm. The supernatant was placed in a vial for chromatography.

Statistical data processing was performed with the help of Microsoft Office Excel 2010.

RESULTS AND DISCUSSION

The affiliation of compounds to the class of flavonoids was evaluated on the basis of absorption spectra. The absorption maxima at 250, 350 ± 5 nm should be observed (as for UV-spectrum of vitexin) to confirm the flavonoid nature (Figure 3).

Calculation of flavonoids content (%) in the PDE was carried out in terms of vitexin according to the formula:

$$X = \frac{S_1 \cdot a_2 \cdot R}{S_2 \cdot a_1}$$

where S_1 – sum of the flavonoid peaks areas on the chromatogram of the test sample; S_2 – peak area of vitexin on the chromatogram of standard sample solution; a_1 – sample mass of PDE, g; a_1 – mass of vitexin in a standard sample solution, g; R – dilution.

Based on the published data²⁴ and taking into account the

Table 1: Scheme of flavonoids gradient elution.

Time	Methanol: acetonitrile (25:75)	0.01% formic acid solution
0	0	100
80	80	20
85	0	100
90	0	100

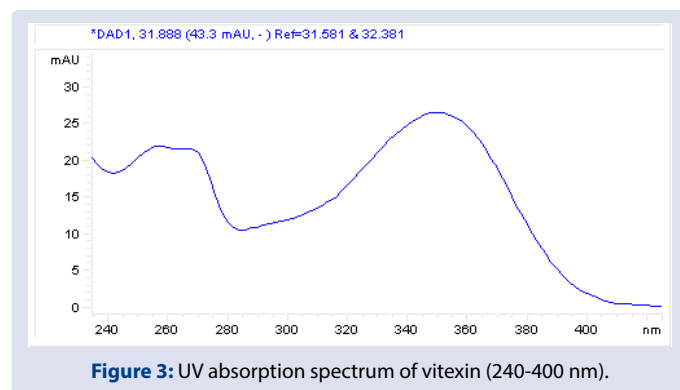


Figure 3: UV absorption spectrum of vitexin (240-400 nm).

chromatographic characteristics of compounds with retention times $R_t = 46.3$ min, $R_t = 55.9$ min, $R_t = 60.99$, it can be assumed that they are probably kaempferitrin, orientin and iso-orientin, respectively. Nevertheless, the data of the MS analysis can reliably confirm these results. Chromatogram obtained in HPLC-UV analysis is presented at Figure 4.

Vitexin is one of the main flavonoids of PDE according to the Table 2.

The results of the flavonoids determination are given in Table 3.

The results of this analysis are consistent with data obtained by other researchers studied *P. incarnata*.²⁴⁻²⁷ The predominant flavonoids are vitexin and orientin. Literature sources display the direct correlation

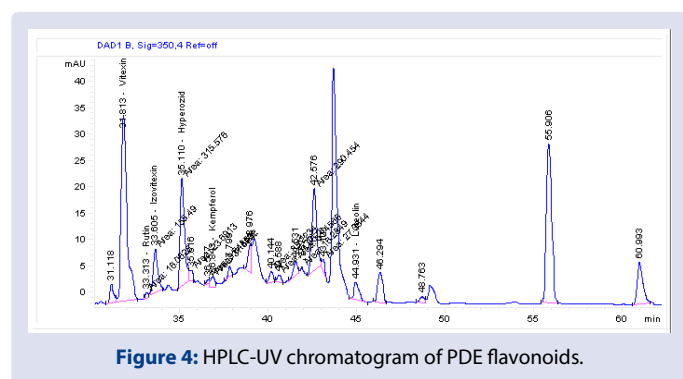


Figure 4: HPLC-UV chromatogram of PDE flavonoids.

Table 2: Characteristics of peaks in the HPLC-UV chromatogram of PDE flavonoids.

No	Retention time (min)	Identified compound	Peak area	% of total peak area	Symmetry coefficient
	31,118		59,2	1,718	0,49
	31,813	vitexin	794,4	23,060	0,592
	33,113	rutoside	16,1	0,467	0,584
	33,605	isovitexin	155,5	4,514	0,728
	35,11	hyperoside	315,6	9,161	0,578
	35,616		23,7	0,688	0,438
	36,577		9,8	0,284	1,534
	36,843	kaempferol	37,9	1,100	0,815
	37,799		28,7	0,833	0,861
	38,976		76	2,206	1,702
	40,144		35,2	1,022	0,858
	40,588		21,7	0,630	0,613
	41,531		51,5	1,495	1,427
	42,576		290,5	8,433	0,859
	43,101		27,1	0,787	0,426
	44,931	luteolin	63,3	1,837	0,551
	46,294	kaempferitrin	143,9	4,177	0,562
	48,763		30,9	0,897	1,003
	55,906	orientin	1026	29,782	0,507
	60,993	isoorientin	238	6,909	0,653

Table 3: The content of vitexin and total flavonoids content in terms of vitexin in PDE.

Object	Number of compounds of flavonoid nature	Vitexin content ($X_{av} \pm \Delta X$), %	Total flavonoids content in terms of vitexin ($X_{av} \pm \Delta X$), %
<i>P. incarnata</i> dry extract	20	0,867 \pm 0,011	3,762 \pm 0,049

N: 5–number of repeat tests; f: 4–number of degrees of freedom; P%: 0.95–confidence figure; t(P,f): 2,78–Student's coefficient; X_{av} : mean value; ΔX : Confidence interval.

between the phenolic compounds quantity and the pharmacological activity.

However, HPLC-UV plays an important role, especially in the qualitative and quantitative analysis of *P. incarnata* flavonoids. The suggested HPLC procedure offers the advantage of using widely available, low-costing analytical standards and can be used in pharmacopoeial analysis of *P. incarnata* medicinal plant materials and herbal drugs.

According to the literature it is clear that *P. incarnata* displays considerable quantitative and qualitative variability of flavonoids, that can be used as markers for herbal extracts. Whereas the BAS responsible for the *P. incarnata* therapeutic effects are yet to be found, herbal drugs should be produced with the approved *P. incarnata* vitexin chemotype until the pharmacological significance of differences in chemotype are found out.

CONCLUSION

In the course of HPLC-UV analysis, it was found that 20 compounds of flavonoid nature are presented in *P. incarnata* dry extract. 9 flavonoids have been identified: isovitexin, vitexin, rutoside, hyperoside, luteolin, kaempferol, kaempferitrin, orientin, and isoorientin. The content of vitexin is $0,867 \pm 0,011\%$, total flavonoids content in terms of vitexin is $3,762 \pm 0,049\%$.

The obtained data will be used for standardization of drugs produced from *P. incarnata* dry extract in the Russian Federation.

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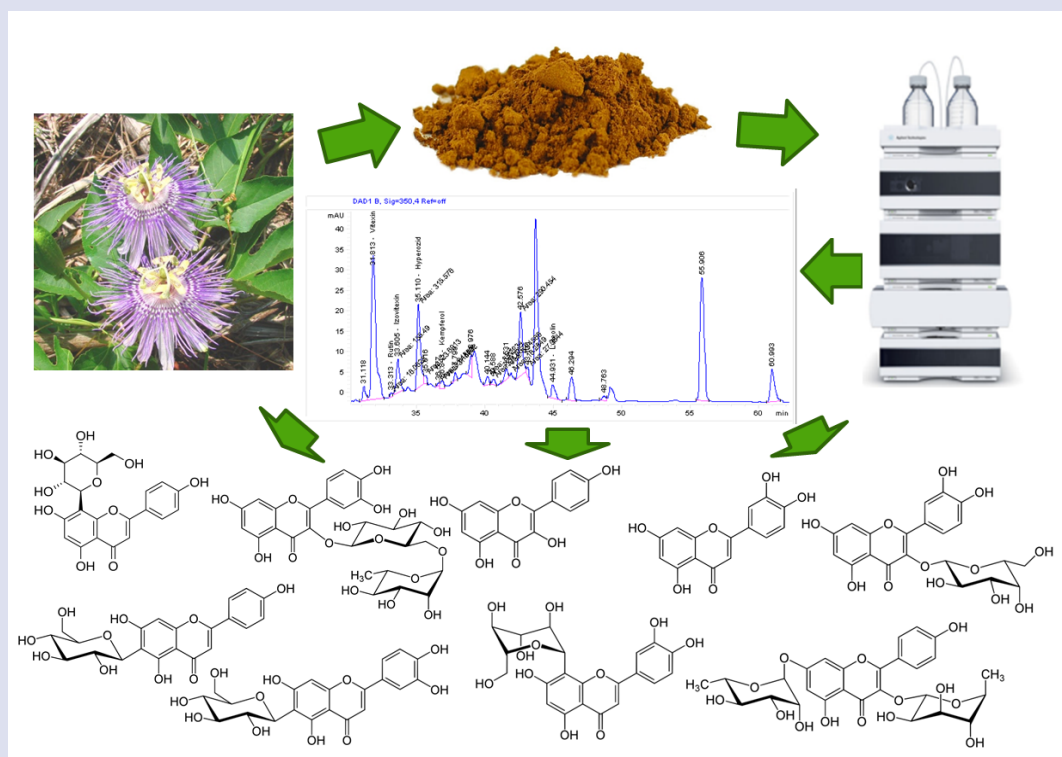
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CONFLICT OF INTERESTS

None.

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