Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Qualitative and quantitative evaluation of the marketing *Passiflora incarnata* L. formulations in Turkey

Dicle ÇEVİK [1] *, Etil GÜZELMERİÇ [2], Nisa Beril ŞEN [2], Erdem YEŞİLADA [2]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Trakya University, Balkan Campus 22030, Edirne, Türkiye.
[2] Department of Pharmacognosy, Faculty of Pharmacy, Yeditepe University, Kayışdağı 34755, İstanbul, Türkiye.

* Corresponding Author. E-mail: dicledemir@windowslive.com (D.Ç.); Tel. +90-284-235-01-40

Received: 13 September 2022 / Accepted: 08 November 2022

ABSTRACT: *Passiflora incarnata* L. (Passifloraceae) has been traditionally used to treat ailments such as dysmenorrhoea, insomnia, epilepsy and neuralgia. In this study, qualitative and quantitative analyses of *P. incarnata* products in the Turkish market were studied via High-Performance Thin-Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC) and vitexin, isovitexin, orientin and isoorientin were used as bioactive marker compounds. Seven herbal tea samples (T1-7) composing *P. incarnata* herb, eighteen capsules/tablets/powders (C1-18) and four syrups (S1-4) containing *P. incarnata* extract were purchased from pharmacies, markets, herbalists or e-commerce websites in Turkey. Comparing HPTLC fingerprinting profiles of the reference plant (*P. incarnata*) with the samples indicated that only T1, C1-C3, C5, C7, C11, C12, C14, C15 and S1 showed similar chemical fingerprints with the reference plant material. On the HPLC analysis, the total ratio of investigated compounds was 6.33% and 2.08% in hydroalcoholic (70% EtOH) and aqueous extracts of reference *P. incarnata*, respectively. Furthermore, hydroalcoholic extract of T1 was found to possess higher content (6.40%) than other tested tea samples (T2-6). However, based on the morphological characteristics, the tea sample (T7) was identified as Jerusalem thorn (*Paliurus spina-christi*) fruits. Among the food supplements (C1-18), only nine were determined to contain the reference molecules (3.301-0.827%), consistent with HPTLC results, and C1 exerted the highest total percentage. To conclude, most of the marketing *P. incarnata* products in Turkey were adulterated or contained lower amounts of bioactive components.

KEYWORDS: *Passiflora incarnata*; Passifloraceae; High-Performance Thin-Layer Chromatography; High-Performance Liquid Chromatography; Quality control.

1. INTRODUCTION

With the recent COVID-19 outbreak, people's psychological health has become threatened with various diseases, such as panic disorder, anxiety, and depression. Thus, the utilization of natural products such as herbal teas, food supplements or traditional herbal medicines having sedative and anxiolytic effects has increased by the public to alleviate these symptoms [1]. On the other hand, maintaining good quality is crucial for reproducible physiological effects and reliability to get the highest benefits from these products. However, due to insufficient control by the authority, adulteration and lack of standardization are considered the main obstacles to natural products [2,3]. Therefore, qualitative and quantitative analysis of herbal medicinal products based on their active or marker component(s) by chromatograpic techniques is very pivotal for the standardization and hence their therapeutic efficacy and safety as well as detection of fraud [4-7].

The genus *Passiflora* (Passifloraceae) is represented by about 520 species around the world grown in warm temperatures and tropical regions such as Central, South or North America, Southeast Asia and Australia [8,9]. Among these, *Passiflora incarnata* L. (passionflower, maypop) is a very popular traditional remedy that has been used in many countries including Turkey, Brasil, India etc. against variety of diseases such as anxiety, stress, insomnia, dysmenorrhea, epilepsy, cancer, neurosis and neuralgia [9-11]. According to The European Medicines Agency (EMA) Committee for Herbal Medicinal Products (HMPC) report, long-term use of passionflower products is effective in reducing mild symptoms of mental stress and aiding sleep [12]. In the "*Passiflorae herba*" monograph of European Scientific Cooperative on Phytotherapy (ESCOP) Monographs, the therapeutic indications of aerial parts of *P. incarnata* containing flowers and/or fruits are tenseness, restlessness and irritability along with difficulty in falling asleep [13].

How to cite this article: Çevik D, Güzelmeriç E, Şen NB, Yelilada E. Qualitative and quantitative evaluation of the marketing Passiflora incarnata L. formulations in Turkey. J Res Pharm. 2023; 27(2): 609-619.

© 2023 Marmara University Press
ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.343

From the phytochemical aspect, flavonoids are reported to be the major components of *P. incarnata* including mainly flavonoid C-glycosides such as vitexin, isovitexin, orientin, isoorientin, schaftoside, and isoschaftoside as well as flavonoid aglycones namely apigenin, luteolin, quercetin, and kaempferol. Besides, low amounts of β-carboline alkaloids (harmane, harmol, harmine), terpenic compounds, and cyanogenic glycosides were also detected [10,14,15]. Previous preclinical studies on the extracts from aerial parts of *P.incarnata* as well as its isolated components revealed a wide range of bioactivity, including anxiolytic, sedative, sleep-enhancing, anticonvulsant and anti-addictive activities [16-22]. *In vivo* studies demonstrated that long-term administration of its hydroalcoholic extract was associated with decreased levels of stress, also improved motor activity and memory functions [23,24]. In clinical practice, a significant number of studies focused on the effectiveness of passionflower extracts, either alone or in combination with other herbal remedies against particularly anxiety, stress and insomnia [25-29]. Studies have shown that bioactive compounds responsible for the sedative and anxiolytic effects of passionflower extracts are mainly C-glycosyl flavonoid derivatives such as vitexin, isovitexin, orientin and isoorientin [30,31]. Therefore, determining its C-glucosyl flavones content as marker components is essential in the quality assessment of the products [32,33].

This study aimed to investigate the quality of *P. incarnata*-containing products, including herbal teas and food supplements marketing in Turkey, by High-Performance Thin-Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC), both qualitatively and quantitatively, based on their bioactive marker compounds of vitexin, isovitexin, orientin and isoorientin.

2. RESULTS

The commercial samples from different brands that contained aerial parts or extracts of *P. incarnata,* including tea samples (T1-6), capsules, tablets or powders (C1-18) and syrups (S1-4) were qualitatively and qualitatively analyzed by HPTLC and HPLC, respectively. C-glucosyl flavones, *i.e.*, vitexin, orientin, isovitexin and isoorientin were used as reference compounds for the quality assessment of these products. Reference plant (*P. incarnata* L.) and tea products (T1-6) were extracted with both H_2O and 70% EtOH to compare the chemical compositions of aqueous and hydroalcoholic extracts from these samples since crude *P. incarnata* materials are mainly used as a tea, tincture or ethanolic extract by the public [34]. On the other hand, among the tea samples, (T7) was identified as Jerusalem thorn (*Paliurus spina-christi*) fruits according to its morphological characteristics and not submitted further chromatographic analysis.

2.1. HPTLC analysis

A standard mixture (std. mix.) containing equal volumes of vitexin, orientin, isovitexin and isoorientin was co-chromatographed with the aqueous (H_2O) and hydroalcoholic (70% EtOH) extracts of reference plant and tea samples (T1-6) as well as other commercial products (C1-18 and S1-4). The identification of reference compounds in std. mix. in samples were confirmed by using R_F values as well as band colors, as shown in Table 1.

Results have demonstrated that either H_2O or 70% EtOH extracts of reference plant material (Ref.) contained all reference compounds in the std. mix. (Figure 1). Comparing HPTLC fingerprinting profiles of reference plant extracts with the H_2O or 70% EtOH extracts of tea samples (T1-6) resulted that only T1 showing a similar chemical profile to reference plant material.

Bright blue zones which were not found in reference plant extracts were detected between $R_F \approx 0.85$-0.90 (after derivatization with NP and PEG captured at 366 nm) in T2-6 extracts (Figure 1). These samples (T2-T6) were products of tea mixtures which contain valerian, lemon balm and chamomile as common ingredients, according to the product labels. Thus, the bright blue zones might be due to the chemical constituents of other plants in these products.

Table 1. R_F values and band colors of investigated compounds by HPTLC after derivatization with NP and PEG, respectively.

Compounds	R_F	Derivatized with NP+ PEG (under 366 nm)
Vitexin	≈0.68	Green
Orientin	≈0.60	Yellow
Isovitexin	≈0.55	Green
Isoorientin	≈0.50	Yellow

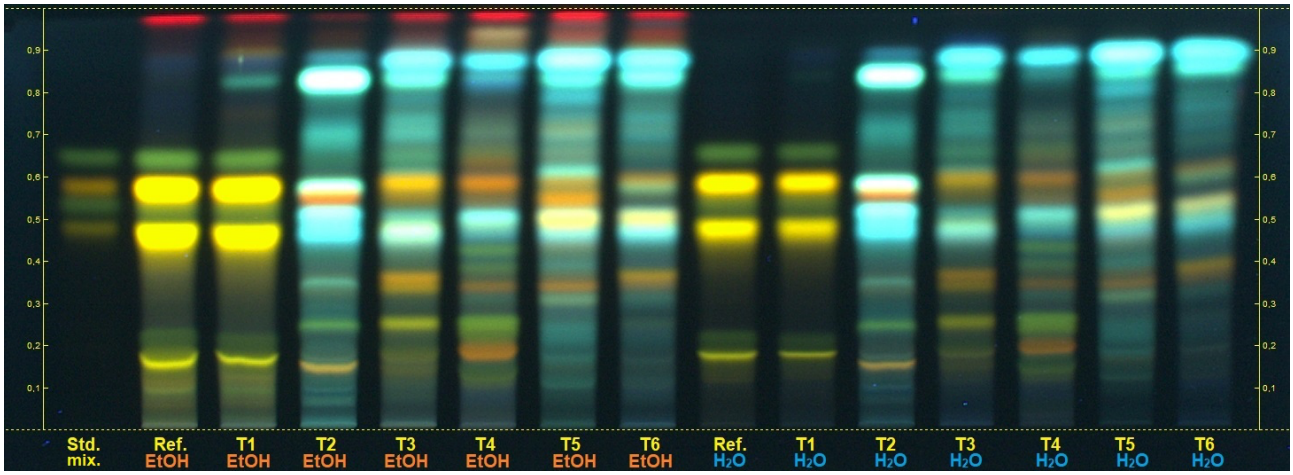

Figure 1. HPTLC chromatogram of hydroalcoholic (EtOH) and aqueous (H_2O) extracts of the reference plant material (Ref.) and herbal tea products (T1-6) sold on the market captured at 366 nm after derivatization with NP and PEG reagents, respectively.

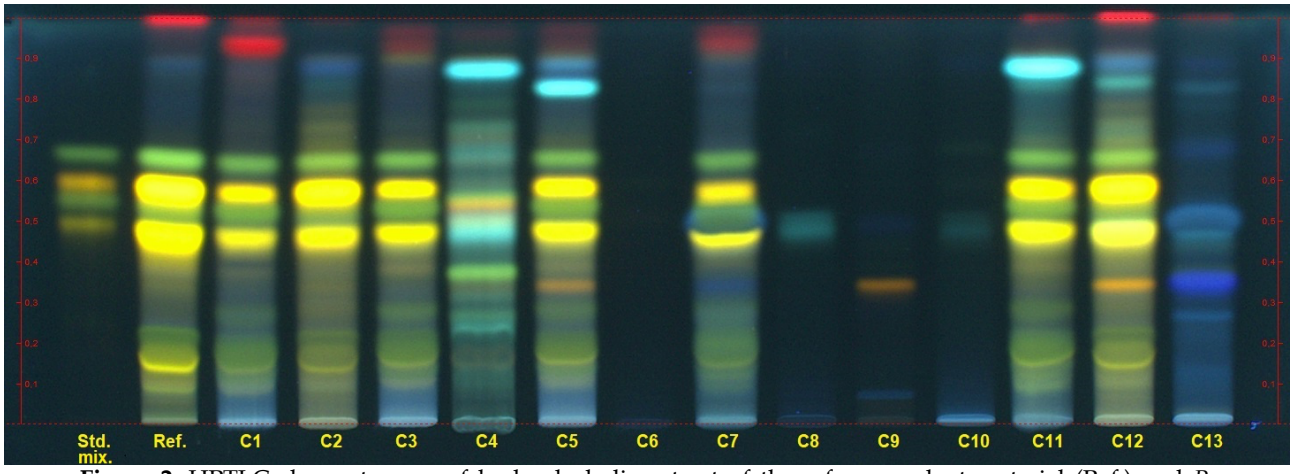

Figure 2. HPTLC chromatogram of hydroalcoholic extract of the reference plant material (Ref.) and *P. incarnata* extract containing products in capsule/tablet forms (C1-13) sold in the market captured at 366 nm after derivatization with NP and PEG reagents, respectively.

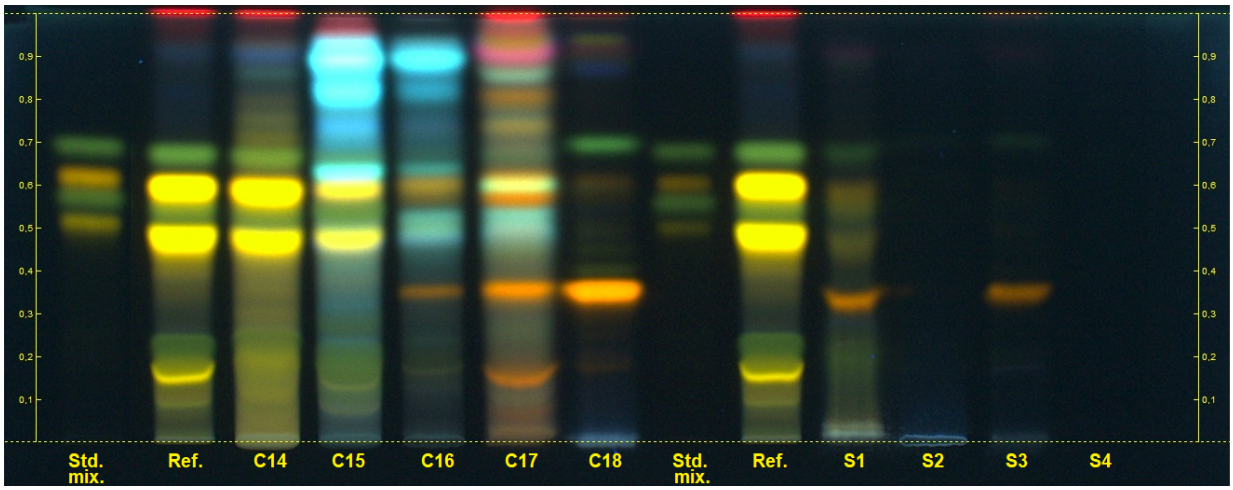

Figure 3. HPTLC chromatogram of hydroalcoholic extract of the reference plant material (Ref.) and *P. incarnata* extract containing products including capsule/tablet/powder (C14-18) and syrups (S1-4) sold in the market captured at 366 nm after derivatization with NP and PEG reagents, respectively.

Eighteen capsule/tablet/powder formulations (encoded C1-18) and four syrups (encoded S1-4) containing *P. incarnata* extract sold in the market were also investigated by HPTLC and their chromatograms were compared with the hydroalcoholic extract of reference *P. incarnata* material (Ref) (Figures 2 and 3). As a

result, reference compounds (vitexin, orientin, isovitexin and isoorientin) in the standard mixture were detected only in C1, C2, C3, C5, C7, C11, C12, C14, C15 and S1.

Observed fade zones on the HPTLC fingerprints of C6, C8, C9, C10 and S2-4, indicating their lower quality. On the other hand, the HPTLC fingerprints of C4, C13, C16, C17 and C18 were utterly different from that of the reference plant extract (Figures 2 and 3). Consequently, these samples (C4, C6, C8-10, C13, C16-18 and S2-4) were considered fraudulent or adulterated.

2.2. HPLC analysis

2.2.1. HPLC method validation

For the quantification of vitexin, orientin, isovitexin and isoorientin, a newly developed HPLC method was validated according to International Conference on Harmonisation (ICH) rules using the specificity, linearity (r^2), detection and determination limits (LOD and LOQ), intraday and inter-days precision, and accuracy (recovery) parameters [35].

In order to evaluate the specificity of the method, the chromatograms of the least concentration of the working standard solutions were comparatively analyzed with the blank chromatogram. As a result, investigated compounds isoorientin t_R = 13.9 ± 0.01, orientin t_R = 14.50 ± 0.01, vitexin t_R = 17.72 ± 0.01 and isovitexin t_R = 18.55 ± 0.01 were not monitored on the blank chromatogram indicating the specificity of the developed method (Figures 4 and 5). In addition, for the detection of the investigated compounds in the samples, UV spectrums of isoorientin and orientin (350 nm), and vitexin and isovitexin (335 nm) were compared with the compounds found in the sample test solutions.

To investigate the linearity, seven different concentration levels (0.5-50 µg/mL) of each freshly prepared working standard solution were analyzed in triplicate. The calibration curve area versus concentration (µg/mL) was found to be linear in the range of 0.5-50 µg/mL with r^2 = 1 for isoorientin, r^2 = 0.9999 for orientin, r^2 = 0.9914 for vitexin and r^2 = 0.9999 for isovitexin as shown in Table 2. LOD and LOQ values were measured from the equation as 3.3 x (SD/S) and 10 x (SD/S), respectively. Accordingly, LOD and LOQ were determined respectively as 0.14 µg/mL and 0.46 µg/mL for isoorientin, 0.45 µg/mL and 1.50 µg/mL for orientin, 0.28 µg/mL and 0.94 µg/mL for vitexin and 0.45 µg/mL and 1.49 µg/mL for isovitexin (Table 2).

The intraday precision of the method was examined by replicating the experiment in triplicate, whereas interday precision was evaluated by repeating the analysis on three consecutive days. The results are presented in Table 3.

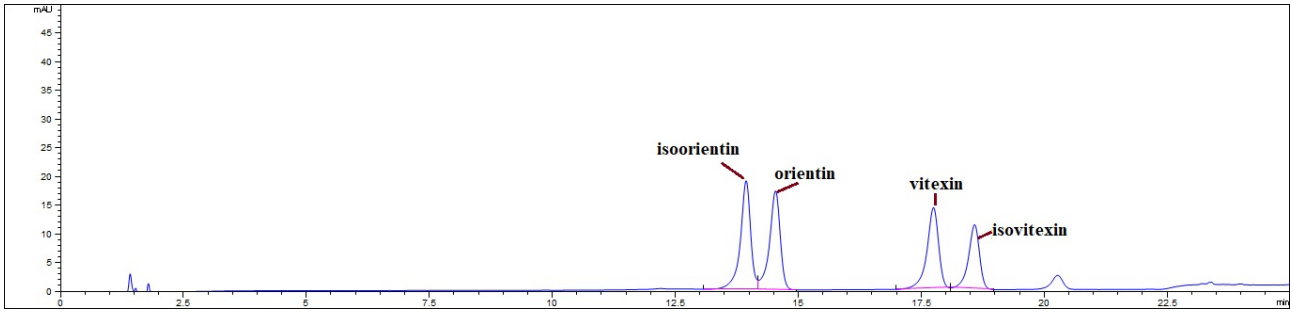

Figure 4 HPLC chromatogram of standard mixture (10 µg/mL) at 350 nm ; isoorientin t_R = 13.9 ± 0.01, orientin t_R = 14.50 ± 0.01, vitexin t_R = 17.72 ± 0.01 and isovitexin t_R = 18.55 ± 0.01

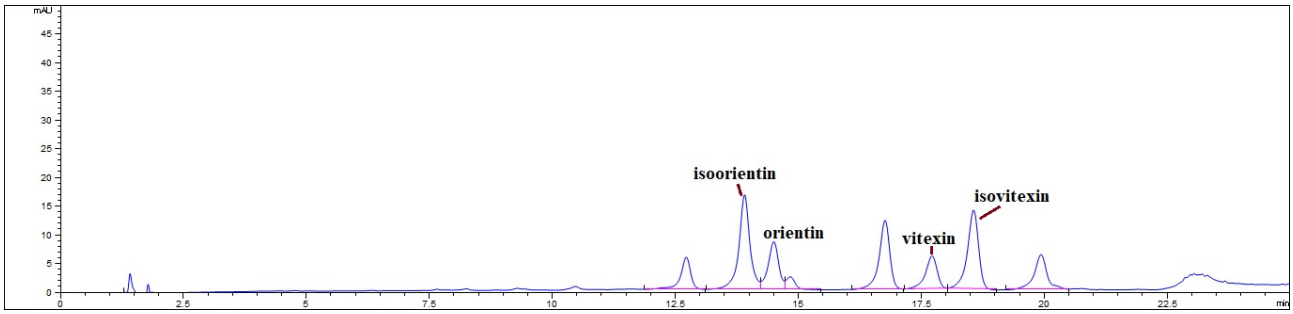

Figure 5. HPLC chromatogram of hydroalcoholic extract of *P. incarnata* (0.5 µg/mL) reference plant material at 350 nm

Table 2. Linearity data of the calibration curves belonging to isoorientin, orientin, vitexin and isovitexin, and LOD and LOQ values.

	Parameters						
Standards	Linearity range µg/mL	r^2	a	b	SD	LOD µg/mL	LOQ µg/mL
Isoorientin	0.5-50	1.0000	27.489	1.631	1.259	0.14	0.46
Orientin	0.5-50	0.9999	25.632	3.057	3.835	0.45	1.50
Vitexin	0.5-50	0.9914	22.696	22.10	2.140	0.28	0.94
Isovitexin	0.5-50	0.9999	18.633	3.895	2.772	0.45	1.49

[a]The calibration equation was 'y=ax+b'.
SD= Standard deviation

Table 3. Repeatability and intra-/interday precision data.

Standards		**Intraday precision**		**Interday precision**		**Interday precision**	
		Average value [a]	**RSD**	**Average value** [a]	**RSD**	**Average value** [a]	**RSD**
Isoorientin	Concentration= 5 µg/mL	4.946± 0.176	3.561	4.990±0.062	1.242	5.043± 0.011	0.208
		5.014± 0.011	0.209				
		4.995± 0.013	0.252				
Orientin		4.883±0.213	4.363	4.949±0.081	1.64	5.038± 0.015	0.293
		4.988±0.002	0.045				
		4.980±0.009	0.181				
Vitexin		4.866±0.198	4.058	4.985±0.067	1.353	5.092±0.004	0.087
		4.997±0.033	0.650				
		4.974±0.009	0.177				
Isovitexin		5.151±0.171	3.315	5.244±0.066	1.258	5.337±0.017	0.323
		5.256±0.030	0.562				
		5.233±0.019	0.355				

[a] The average values were expressed as µg/mL±SD; *n*=3.

Table 4. Recovery results.

Standards	**Theoretical value** [a]	**Amount found** [b]	**Recovery (%)**	**RSD**
Isoorientin	3.125	3.331±0.065	106.592	1.958
	6.25	6.175±0.236	98.793	3.830
	12.5	11.840±0.232	94.719	1.695
Orientin	3.125	3.184±0.082	101.880	2.589
	6.25	6.097±0.281	97.548	4.607
	12.5	11.838±0.288	94.706	2.437
Vitexin	3.125	2.740±0.065	87.669	2.380
	6.25	6.056±0.168	96.895	2.770
	12.5	13.275±0.221	106.196	1.668
Isovitexin	3.125	2.954±0.053	94.527	1.783
	6.25	6.018±0.188	96.295	3.121
	12.5	11.707±0.203	93.658	1.734

[a]Theoretical value of the standards were expressed as µg/mL.
[b]The average found amounts were expressed as µg/mL±SD, *n*=3.
RSD: Relative standard deviation

Recovery was determined by the ratio in per cent between the known spiked amount of the compound and the experimentally found result. The three different concentrations of standard compounds (3.125, 6.25 and 12.5 µg/mL) were spiked into the previously analyzed sample test solution. The results showed that the developed method has a good recovery rate from 94.719 to 106.592% for isoorientin, 94.706 to 101.880% for orientin, from 87.669 to 106.196% for vitexin and from 93.658 to 96.295% for isovitexin as depicted in Table 4.

2.2.2. Quantitative analysis of reference compounds

Marker compounds, including vitexin, orientin, isovitexin and isoorientin (mg/g) in the reference *P. incarnata* extracts and marketed products (T1-6 and C1-18) were quantitatively analyzed by HPLC (Table 5 and 6). Table 5 shows that neither the H_2O nor the 70% EtOH of T2, T4-T6 contained the reference compounds. On the other hand, in the H_2O and 70% EtOH extracts of sample T3, isoorientin, orientin and isovitexin were detected, whereas vitexin was absent.

A comparison of the H_2O and 70% EtOH extracts of the reference plant material and T1 indicated a remarkable increase in the amounts of all investigated compounds (vitexin, orientin, isovitexin and isoorientin) in the 70% EtOH extract. Namely, the increase was from 5.76±0.01 to 19.3±0.40 mg/g in isoorientin; from 3.25±0.01 to 9.9±0.20 mg/g in orientin; from 3.16±0.01 to 8.1±0.10 mg/g in vitexin and from 8.65±0.01 to 26.0±0.40 mg/g in isovitexin contents respectively, in the H_2O and 70% EtOH extracts obtained from the reference plant material. Amongst the tea samples, H_2O and 70% EtOH extracts of T1 were found to have the highest content with the concentrations of isoorientin [3.71±0.15 and 17.50±0.00 mg/g], orientin [2.21±0.1 and 9.85±0.01 mg/g], vitexin [1.88±0.05 and 8.50±0.04 mg/g] and isovitexin [6.26±0.17 and 28.19±0.03 mg/g], respectively.

Furthermore, we compared the total percentage of the investigated bioactive C-glucoside flavonoids to those in the samples. As a result, the total percentages of investigated reference compounds were found to be 6.33% and 2.08% in 70% EtOH and H_2O extracts of the reference *P. incarnata* material, respectively. In comparison, these values were calculated as 6.40% and 1.41%, respectively, for 70% EtOH and H_2O extracts of sample T1. In sample T3, the total percentage of reference compounds was found to be relatively low (0.97% and 0.48%). This might be because it is in a mixture of tea formulation and composing *P. incarnata* in very low amounts.

Amongst capsule/tablet/powder samples (C1-18) that were claimed to contain *P. incarnata* extracts, only C1-3, C5, C7, C11, C12, C14, and C15 were found to comprise the investigated reference compounds between 3.301-0.827%, whereas C4, C6, C8-10, C13 and C16-18 were devoid any of them (Table 6). The highest total percentage of reference compounds was found in sample C1 (3.301%) which was followed by C3 (2.124%).

Table 5. Isoorientin, orientin, vitexin and isovitexin contents (mg/g) in aqueous and hydroalcoholic extracts of reference plant *P. incarnata* (Ref.) and tea samples (T1-6).

	Products	Isoorientin (mg/g±SD)	Orientin (mg/g±SD)	Vitexin (mg/g±SD)	Isovitexin (mg/g±SD)	Total percentage (%) of references
***P. incarnata* aqueous ext.**	**Ref.** H_2O	5.76±0.01	3.25±0.01	3.16±0.01	8.65±0.01	2.08%
H_2O extracts of the tea samples	**T1** H_2O	3.71±0.15	2.21±0.1	1.88±0.05	6.26±0.17	1.41%
	T2 H_2O	nd	nd	nd	nd	-
	T3 H_2O	1.66±0.04	0.71±0.02	nd	2.40±0.03	0.48%
	T4 H_2O	nd	nd	nd	nd	-
	T5 H_2O	nd	nd	nd	nd	-
	T6 H_2O	nd	nd	nd	nd	-
***P. incarnata* hydroalcoholic ext.**	**Ref.** EtOH	19.3±0.40	9.9±0.20	8.1±0.10	26.0±0.40	6.33%
70% EtOH extracts of tea samples	**T1** EtOH	17.50±0.00	9.85±0.01	8.50±0.04	28.19±0.03	6.40%
	T2 EtOH	nd	nd	nd	nd	-
	T3 EtOH	3.05±0.07	1.54±0.07	nd	5.12±0.13	0.97%
	T4 EtOH	nd	nd	nd	nd	-
	T5 EtOH	nd	nd	nd	nd	-
	T6 EtOH	nd	nd	nd	nd	-

Results are expressed as mean ± Standard deviation (SD)
nd: Not detected

Table 6. Isoorientin, orientin, vitexin and isovitexin contents (mg/g) in marketing food supplement products containing *P. incarnata* extracts (C1-18)

Products	Isoorientin (mg/g±SD)	Orientin (mg/g±SD)	Vitexin (mg/g±SD)	Isovitexin (mg/g±SD)	Total percentage (%) of references
C1	10.07±0.41	0.78±0.04	2.50±0.11	19.66±0.53	3.301%
C2	2.08±0.04	1.88±0.04	1.46±0.03	2.85±0.04	0.827%
C3	6.33±0.10	0.71±0.02	1.58±0.03	12.63±0.12	2.124%
C4	nd	nd	nd	nd	-
C5	5.83±0.24	0.66±0.09	1.25±0.04	11.37±0.22	1.912%
C6	nd	nd	nd	nd	-
C7	3.85±0.08	0.21±0.00	0.33±0.03	7.80±0.10	1.219%
C8	nd	nd	nd	nd	-
C9	nd	nd	nd	nd	-
C10	nd	nd	nd	nd	-
C11	6.43±0.30	0.85±0.05	1.12±0.1	11.28±0.32	1.968%
C12	2.49±0.13	1.79±0.1	1.49±0.07	4.21±0.16	0.999%
C13	nd	nd	nd	nd	-
C14	4.65±0.20	3.41±0.17	3.08±0.09	7.09±0.22	1.514%
C15	5.42±0.21	0.62±0.04	1.43±0.11	12.98±0.42	1.902%
C16	nd	nd	nd	nd	-
C17	nd	nd	nd	nd	-
C18	nd	nd	nd	nd	-

3. DISCUSSION

A complete fraud was detected in one of the purchased tea samples (T7), and the plant material was identified as Jerusalem thorn (*Paliurus spina-christi* Mill.- Rhamnaceae) fruit based on its morphological characteristics. This finding was found consistent with the study of Köse and Köroğlu (2021), where 2 of 3 samples that were sold under the name of "passionflower" or "passiflora" were determined to be *P. spina-christi* fruits [36].

Concurrent use of HPTLC and HPLC techniques offered many advantages in the quality assessment of herbal drugs. Because HPTLC results might be deceptive in qualitative analysis of the mixture samples possessing more than one herbal drug or extract. Some of the analyzed marketed formulations claimed to contain *P. incarnata* were a mixture of various herbal drugs (T2-6) or extracts (C4 and C6-17). Hence, comparing their chemical fingerprints only by HPTLC was not sufficient to decide their quality whether these products were adulterated or of low quality. Through HPLC-DAD detectors, flavonoid peaks can be easily identified with two UV absorption peaks in 240-400 nm region [32]. Therefore, HPLC was also helpful in confirming our HPTLC results besides quantification of the components. The findings of both techniques were in good agreement except the sample T3. Although it contained isoorientin (1.66±0.04 and 3.05±0.07 mg/g), orientin (0.71±0.02 and 1.54±0.07 mg/g), and isovitexin (2.40±0.03 and 5.12±0.13 mg/g) in its H_2O and 70% EtOH extracts, respectively, a different chemical profile than the reference plant extract was observed (Figure 1 and Table 5) .

European Pharmacopoeia suggests a spectrophotometric method to determine the total flavonoid content, calculated as vitexin percentage and its minimum limit was set 1.5% in "*Passiflorae Herba*" and 2.0% in "*Passiflorae Herbae Extractum Siccum*" monographs [37]. However, HPLC is recognized as more beneficial in quantitative analysis in terms of specified and accurate results, particularly for evaluating the mixture samples. Therefore, a HPLC method was developed and validated for quality analysis of *P. incarnata*-containing products.

It is noteworthy to mention that isovitexin (2.40±0.03-28.19±0.03 mg/g) was found to be a relatively more abundant component than the other investigated reference compounds, and this followed by isoorientin (1.66±0.04-19.3±0.4 mg/g) in the investigated samples (Table 5 and 6). Similarly, Avula et al. (2012) also

detected isovitexin (0.38% and 0.67%, w/w) and isoorientin (0.11% and 0.34%, w/w) as major components among the other C-glycosides (vitexin and orientin) in two of each plant materials from the aerial parts of *P. incarnata* by HPLC [15], supporting our results.

Flavonoid contents in passionflower samples may vary depending on several factors, including climate, locality, collection period of the year, storage conditions and duration, as well as the extraction procedures. As reported by Menghini and Mancini (1988), the isovitexin production of *P. incarnata* L. has reached its maximum level between the pre-flowering and flowering stages, and the highest flavonoid accumulation was detected in the leaves. Meanwhile, total flavonoid content decreased by 60% one year after harvesting [33]. In our study, the yield of marker compounds in the aqueous extracts from passionflower tea samples (Ref: 2.08% and T1: 1.41%) was found to be lower than their ethanolic extracts (Ref: 6.33% and T1: 6.40%) (Table 5). In a previous study by Masteikova et al. (2008), quantitative data by HPLC revealed dramatically higher values of flavonoids, including isovitexin, vitexin, orientin, hyperoside, quercetin, luteolin, rutin, vicenin in the ethanolic (70%) extract of *P. incarnata* comparing to its aqueous extract [34]. So, these aforementioned procedures must be given attention by the producers.

In Turkey, herbal medicinal products are authorized either by the Ministry of Health as "Traditional Herbal Medicinal Products" or by the Ministry of Agriculture and Forestry as "Food Supplements". Sample C1, which was the only Ministry of Health licensed product, was found to possess relatively best quality with a total 3.301% of standard compounds than the other extract-containing samples licenced as food supplements (C2-18) (Table 6). This is a significant finding that points out the importance of the requirements in the authorization processes of phytomedicines.

This study is the first report comparatively evaluating the fingerprinting and quantitative analysis of the marketed passionflower products in Turkey by HPTLC and HPLC techniques.

4. CONCLUSION

As a result of the chemical fingerprinting analysis in marketed tea (T1-6), food supplements in capsule/tablet/powder (C1-18) and syrup (S1-4) formulations composing aerial parts or extracts of *P. incarnata* by using HPTLC method along with vitexin, isovitexin, orientin and isoorientin as bioactive marker compounds; only one of the tea samples (T1) and some of the food supplements (C1-C3, C5, C7, C11, C12, C14, C15 and S1) showed similar chemical profile with reference plant material (*P. incarnata*). On the other hand, fraud was detected in a tea sample T7, identified as Jerusalem thorn fruits. Further HPLC analysis for the quantification of marker compounds showed that hydroalcoholic (70% EtOH) extract of T1 (6.40%) was found to possess the best quality among the other tested tea samples (T2-6). In contrast, the investigated reference compounds (3.301-0.827%) were detected only in nine out of eighteen food supplements encoded as C1-18, supporting HPTLC results. Among the formulations containing *P. incarnata* extract, C1, licenced by the Ministry of Health as a "Traditional Herbal Medicinal Product" was found to possess relatively best quality with a total of 3.301% content of standard compounds than the other samples (C2-18) licenced as "Food Supplements". To conclude, most of the products marketed in Turkey are labelled to contain *P. incarnata* herb, or its extract was determined to be adulterated or with low amounts of bioactive components.

5. MATERIALS AND METHODS

5.1. Chemicals and solvents

HPLC grade acetonitrile and methanol were acquired from Sigma-Aldrich (Steinheim, Germany). Formic acid, methyl ethyl ketone, ethyl acetate, phosphoric acid, and ethanol were purchased from Sigma-Aldrich (Steinheim, Germany). 2-Aminoethyl diphenylborinate was from Fluka (Steinheim, Germany). Polyethylene glycol 400 was obtained from Merck (Darmstadt, Germany). The ultrapure water was obtained from Millipore, Simplicity UV (Darmstadt, Germany). Reference compounds of vitexin, orientin and isoorientin were purchased from Sigma-Aldrich (Steinheim, Germany), while isovitexin was isolated from *Vitex agnus-castus*, previously [38].

5.2. Plant material and samples containing *Passiflora incarnata*

Aerial parts of *P.incarnata* were collected from a cultivar in Muğla (Turkey) during its flowering season and assigned as reference plant material. Seven herbal tea products (T1-7) containing *P. incarnata*, eighteen samples in capsule, tablet or powder forms (C1-18), and four samples in syrup forms (S1-4) containing *P. incarnata* extract were provided from pharmacies, markets, herbalists or e-commerce websites in Turkey. Each product was sold under a different brand name, while some (T2-T6, C4 and C6-17) were a mixture of other herbal drugs such as valerian (*Valeriana officinalis*), lemonbalm (*Melissa officinalis*) and/or chamomile

(*Matricaria* sp.) etc. The amount of *P. incarnata* extract declared on the labels of these commercial formulations ranged from 75 to 525 mg. Only 2 of these products (C1 and S1) were registered by the Ministry of Health as "Traditional Herbal Medicinal Product" and were only available in pharmacies.

5.3. Preparation of standard solutions

5.3.1. Standard solutions for HPTLC analysis

Standard solutions of vitexin, orientin, isovitexin and isoorientin were prepared in methanol at 0.2 mg/mL concentration. Equal volumes of vitexin, orientin, isovitexin and isoorientin standard solutions were mixed in order to prepare a standard mixture (std mix).

5.3.2. Standard solutions for HPLC analysis

Stock solutions of vitexin, orientin, isovitexin and isoorientin (1000 µg/mL) were prepared in methanol and also diluted to obtain a calibration curve and to perform recovery studies.

5.4. Preparation of sample test solutions

5.4.1. Extraction of P. incarnata as a reference plant material

Reference plant material *P. incarnata* aerial parts was extracted with either water or 70% ethanol to obtain ref. H_2O and ref. 70% EtOH extracts, respectively. For water extraction, an infusion method was applied. Accurately weighed 2 g of powdered plant material was infused with 100 mL of boiling water. Following 15 minutes of brewing, it was filtered through Whatman paper and lyophilized (Yield=36.6%). The Sonication technique was applied for the hydroalcoholic extraction of reference plant material. 1 g accurately weighed powdered plant material was extracted with 10 mL 70% ethanol in a sonicator for 30 minutes, followed by filtration and evaporated to dryness under reduced pressure (Yield=11.8%). The reference plant material's water and hydroalcoholic extracts were diluted to 50 mg/mL in MeOH and filtered by a syringe filter (0.45 µm) to obtain the reference test solutions.

5.4.2. Extraction of the marketed tea products

Tea samples which were encoded as T1-T6 were randomly chosen for homogeneous sampling, weighed precisely and then subjected to infusion or hydroalcoholic extraction according to aforementioned procedure in *section 5.4.1.* The water and hydroalcoholic extracts were then diluted with MeOH to 50 mg/mL and filtered by a 0.45 µm syringe filter for further analysis. On the other hand, T7 was not extracted and applied to further chromatographic analyses due to the detection of fraud concerning its morphological incompatibility with *P. incarnata*.

5.4.3. Sample preparation for the extract-containing products

Following the selection of six capsules or tablets randomly for homogeneous sampling, samples were powdered and weighed, and the final concentrations of *P. incarnata* extracts in each sample test solutions of C1-18 were adjusted to 50 mg/mL in MeOH according to declared amounts per capsule or tablet of the product labels. Similarly, concentrations of syrups were adjusted to 30 mg/mL in MeOH. The yielded test solution samples were finally filtered through a syringe filter (0.45 µm) for further analysis.

5.5. HPTLC method

Reference plant material (2 µL), sample test solutions of marketed samples, including tea (2 µL), capsules, tablets, powders (2 µL) or syrups (10 µL), as well as the mixture of standard solution (2 µL) were applied as bands using Linomat V semi-automatic sample applicator (Camag, Muttenz, Switzerland) coupled with 100 µL Hamilton syringe on HPTLC plate (silica gel 60 F_{254} glass plate, 20 x 10 cm). Anhydrous formic acid-H_2O-methyl ethyl ketone-ethyl acetate (10:10:30:50, *v/v/v/v*) was used as the mobile phase. The plates were then developed up to 7 cm in 20 minutes saturated twin-trough chamber (Camag). After the development process, derivatization was applied first by heating the plate at 100˚C on the Camag TLC plate heater for 3 minutes and then by dipping with Natural Product reagent (NP reagent) and PEG 400 solutions in TLC Immersion III (Camag) tool, respectively. Finally, Camag TLC visualizer was used for the documentation of the plates at 366 nm.

5.6. HPLC method

HPLC analysis was performed by using Agilent 1260 Infinity HPLC system (Darmstadt, Germany) consisting of an Agilent ChemStation software, quaternary pump (G1311B), auto-sampler (G1329B), thermostatted column compartment (G1316A), and diode array detector (G4212B). The Agilent Pursuit 5 RP18 Column (3.9 mm x 150 mm, 5-µm particle size) was used at 25 ˚C to separate the reference compounds. The gradient elution was used by using mobile phases A (*o*-phosphoric acid-water (0.1:99.9, *v/v*) and B (acetonitrile). The following gradient system was applied: 5-10%B (0-2 min.), 10-16% B (2-20 min.), 16-80% B (20-23 min.), 80% B (23-24 min.) and 80-5% B (24-28 min.). 10 µL of the standard and test solutions were injected into the system. The flow rate was 1 mL/min. Isoorientin and orientin were monitored at 350 nm, while isovitexin and vitexin were detected at 335 nm. This newly developed method was validated following the International Conference on Harmonisation (ICH) 1995 guidelines [35].

Acknowledgements: This work was supported by the Scientific Research Project Fund of Trakya University under project number 2021/35.

Author contributions: Concept – D.Ç., E.G.; Design – D.Ç., E.G., E.Y.; Supervision – E.Y.; Resources – D.Ç., E.G., E.Y.; Materials – D.Ç., E.G., E.Y.; Data Collection and/or Processing – D.Ç., E.G, N.B.Ş.; Analysis and/or Interpretation – D.Ç., E.G., N.B.Ş.; Literature Search – D.Ç., E.G., N.B.Ş., E.Y.; Writing – D.Ç., E.G., N.B.Ş., E.Y.; Critical Reviews – D.Ç., E.G., N.B.Ş., E.Y.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Qiu J, Shen B, Zhao M, Wang Z, Xie B, Xu Y.A nationwide survey of psychological distress among Chinese people in the COVID-19 epidemic: Implications and policy recommendations. Gen Psychiatry. 2020; 33: 19–21. https://doi.org/10.1136/gpsych-2020-100213
[2] Chanda S. Importance of pharmacognostic study of medicinal plants: An overview. J Pharmacogn Phytochem. 2014; 2: 69–73.
[3] Calahan J, Howard D, Almalki AJ, et al. Chemical Adulterants in Herbal Medicinal Products: A Review. Planta Med. 2016; 82: 505–515. https://doi.org/10.1055/s-0042-103495
[4] Deconinck E, De Leersnijder C, Custers D, Courselle P, De Beer JO. A strategy for the identification of plants in illegal pharmaceutical preparations and food supplements using chromatographic fingerprints. Anal Bioanal Chem. 2013; 405: 2341–2352. https://doi.org/10.1007/s00216-012-6649-4
[5] Dişli M, Yeşilada E. Türkiye 'de Bitkisel Tıbbi Ürünler (Türkiye'de Bitkisel Ürünlerin Standardizasyonu, Üretimi Ve Tağşiş) : Derleme. J Biotechnol Strateg Heal Res. 2019; 3: 13–21. https://doi.org/10.34084/bshr.545498
[6] Güzelmeriç E, Çevik D, Yeşilada E. Quality assessment of marketed food supplements and herbal tea products of olive leaf in Turkey. J Res Pharm. 2020; 24: 159–169. https://doi.org/10.35333/jrp.2020.123
[7] Güzelmeriç E, Çelik C, Şen NB, Oçkun MA, Yeşilada E. Quali/quantitative research on herbal supplements containing black elder (*Sambucus nigra* L.) fruits. J Res Pharm. 2021; 25: 238–248. https://doi.org/10.29228/jrp.14
[8] Ulmer T, John Mochrie M. Passiflora : Passionflowers of the world, Timber Press, Portland 2004.
[9] Miroddi M, Calapai G, Navarra M, Minciullo PL, Gangemi S. *Passiflora incarnata* L.: Ethnopharmacology, clinical application, safety and evaluation of clinical trials. J Ethnopharmacol. 2013; 150: 791–804. https://doi.org/10.1016/j.jep.2013.09.047
[10] Dhawan K, Dhawan S, Sharma A. Passiflora: A review update. J Ethnopharmacol. 2004; 94: 1–23. https://doi.org/10.1016/j.jep.2004.02.023
[11] Taylor L, Herbal Secrets of the Rainforest, Prime Publishing Inc, Austin, TX, 1996.
[12] Committee for Herbal Medicinal Products (HMPC) Assessment Report on *Passiflora incarnata* L. https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-passiflora-incarnata-l-herba_en.pdf (accessed on 11 September 2022).
[13] European Scientific Cooperative on Phytotherapy E.S.C.O.P. Monographs, second ed., Thieme, New York, 2003, pp. 539–546.
[14] Barnes J, Anderson LA, Phillipson JD, Herbal medicines, third ed., Pharmaceutical press, 2007.
[15] Avula B, Wang YH, Rumalla CS, Smillie TJ, Khan IA. Simultaneous determination of alkaloids and flavonoids from aerial parts of *Passiflora* species and dietary supplements using UPLC-UV-MS and HPTLC. Nat Prod Commun. 2012; 7: 1177–1180. https://doi.org/10.1177/1934578x1200700918
[16] Speroni E, Minghetti A. Neuropharmacological activity of extracts from *Passiflora incarnata*. Planta Med. 1988; 54: 488–491. https://doi.org/10.1055/s-2006-962525
[17] Dhawan K, Kumar S, Sharma A. Anti-anxiety studies on extracts of *Passiflora incarnata* Linneaus. J Ethnopharmacol. 2001; 78: 165–170. https://doi.org/10.1016/S0378-8741(01)00339-7
[18] Dhawan K, Kumar S, Sharma A. Comparative anxiolytic activity profile of various preparations of *Passiflora incarnata* Linneaus. J Altern Complemantary Med. 2002; 8: 283–291. https://doi.org/10.1089/10755530260127970

[19] Dhawan K. Drug/substance reversal effects of a novel tri-substituted benzoflavone moiety (BZF) isolated from *Passiflora incarnata* Linn. - A brief perspective. Addict Biol. 2003; 8: 379–386. https://doi.org/10.1080/1355621031000164638S
[20] Dhawan K, Kumar S, Sharma A. Evaluation of central nervous system effects of *Passiflora incarnata* in experimental animals. Pharm Biol 2003; 41: 87–91. https://doi.org/10.1076/phbi.41.2.87.14241
[21] Nassiri-Asl M, Shariati-Rad S, Zamansoltani F. Anticonvulsant effects of aerial parts of *Passiflora incarnata* extract in mice: Involvement of benzodiazepine and opioid receptors. BMC Complement Altern Med. 2007; 7: 3–8. https://doi.org/10.1186/1472-6882-7-26
[22] Grundmann O, Wähling C, Staiger C, Butterweck V. Anxiolytic effects of a passion flower (*Passiflora incarnata* L.) extract in the elevated plus maze in mice. Pharmazie. 2009; 64: 63–64. https://doi.org/10.1691/ph.2008.8753
[23] Jawna-zboi K, Blecharz-klin K, Joniec-maciejak I, Wawer A, Pyrzanowska J, Piechal A, Mirowska-guzel D. *Passiflora incarnata* L . improves spatial memory, reduces stress and affects neurotransmission in rats. Phytother Res. 2016; 30 (5): 781–789. https://doi.org/10.1002/ptr.5578
[24] Kim G, Lim K, Sung H, Lee J, Kim Y, Park S. Improvement in neurogenesis and memory function by administration of *Passiflora incarnata* L . extract applied to sleep disorder in rodent models. J Chem Neuroanat. 2019; 98: 27–40. https://doi.org/10.1016/j.jchemneu.2019.03.005
[25] Akhondzadeh S, Naghavi HR, Vazirian M, Shayeganpour A, Rashidi H, Khani M. Passionflower in the treatment of generalized anxiety: A pilot double-blind randomized controlled trial with oxazepam. J Clin Pharm Ther. 2001; 26: 363–367. https://doi.org/10.1046/j.1365-2710.2001.00367.x
[26] Movafegh A, Alizadeh R, Hajimohamadi F, Esfehani F, Nejatfar M. Preoperative oral *Passiflora incarnata* reduces anxiety in ambulatory surgery patients: A double-blind, placebo-controlled study. Anesth Analg 2008; 106: 1728–1732. https://doi.org/10.1213/ane.0b013e318172c3f9
[27] Ngan A, Conduit R. A double-blind, placebo-controlled investigation of the effects of *Passiflora incarnata* (Passion flower) herbal tea on Subjective Sleep Quality. Phytother Res., 2011; 25(8): 1153–1159. https://doi.org/10.1002/ptr.3400
[28] Aslanargun P, Cuvas O, Dikmen B, Aslan E, Yuksel MU. *Passiflora incarnata* Linneaus as an anxiolytic before spinal anesthesia. J Anesth. 2012; 26: 39–44. https://doi.org/10.1007/s00540-011-1265-6
[29] Janda K, Wojtkowska K, Jakubczyk K, Antoniewicz J, Skonieczna-Żydecka K. *Passiflora incarnata* in neuropsychiatric disorders-A systematic review. Nutrients. 2020; 12(12): 3894. https://doi.org/10.3390/nu12123894
[30] Alves JSF, Marques JI, Demarque DP, Costa LRF, Amaral JG, Lopes NP, Silva-Júnior AA, Soares LAL, Gavioli EC, Ferreira LDS, Zucolotto SM. Correction to: Involvement of isoorientin in the antidepressant bioactivity of a flavonoid-rich extract from *Passiflora edulis* f. *flavicarpa* leaves. Rev Bras Farmacogn. 2020; 30: 319. https://doi.org/10.1007/s43450-020-00051-3
[31] He M, Min J-W, Kong W-L, He X-H, Li J-X, Peng B-W. A review on the pharmacological effects of vitexin and isovitexin. Fitoterapia. 2016; 115: 74–85. https://doi.org/10.1016/j.fitote.2016.09.011
[32] Pereira CAM, Yariwake JH, Lanças FM, Wauters J-N, Tits M, Angenot L. A HPTLC densitometric determination of flavonoids from *Passiflora alata, P. edulis, P. incarnata* and *P. caerulea* and comparison with HPLC Method. Phytochem Anal. 2004; 15: 241–248. https://doi.org/10.1002/pca.778
[33] Menghini A, Mancini LA. TLC determination of flavonoid accumulation in clonal populations of *Passiflora incarnata* L. Pharmacol Res Commun. 1988; 20: 113–116. https://doi.org/10.1016/S0031-6989(88)80854-3
[34] Masteikova R, Bernatoniene J, Bernatoniene R, Velziene S. Antiradical activities of the extract of *Passiflora incarnata*. Acta Pol Pharm - Drug Res. 2008; 65: 577–583.
[35] ICH Harmonised Tripartite Guideline Validation of Analytical Procedures: Text and Methodology Q2 (R1). https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf (accessed on 11 September 2022).
[36] Köse EC, Köroğlu A. *Passiflora* genus, its importance of pharmacy and investiigation of the samples which is sold by passiflora (çarkıfelek) name in the market of Turkey. Biol Divers Conserv. 2021; 14: 138–145. https://doi.org/10.46309/biodicon.2021.814383
[37] European Pharmacopoeia (Ph. Eur.), seventh ed., Council of Europe, Strasbourg, France, 2010.
[38] Kırmızıbekmez H, Demir D. Iridoid glycosides and phenolic compounds from the flowers of *Vitex agnus-castus*. Helv Chim Acta. 2016; 99: 518–522. https://doi.org/10.1002/hlca.201600022

This is an open-access article which is publicly available on our journal's website under Institutional Repository at http://dspace.marmara.edu.tr.