Variation of parthenolide and phenolic compounds content in different parts of *Tanacetum parthenium* (L.) Schulz Bip., Asteraceae during 18 months storage

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Feverfew (*Tanacetum parthenium* (L.) Schulz Bip., Asteraceae), a medicinal plant with a long tradition in European folk medicine, has been used to treat headache, fever, rheumatism, asthma, stomach pains, and other conditions related to inflammation. Although feverfew is often used for migraine headaches, numerous literature data stated the inconclusiveness regarding its efficacy. Namely, there is the discrepancy between the stated and the found quantity of the carrier of the mentioned activity (parthenolide) in the herbal products containing feverfew or its extracts. Based on the EMA monographs, it is evident that the feverfew phytopreparation might be designed from the raw herbal material, adequately prepared. Therefore, in this work we investigated the variation of the parthenolide content in different parts of this plant during 18 months storage in order to enable the data regarding the possible use of the powered plants in preparations aimed for migraine prophylaxis. Besides, the total phenolic compound content was determined, as well. The results revealed that the content of parthenolide decreased during the investigated period. In contrast, parthenolide content in the commercially available extract did not change during the time of investigation. Different particle size of the tested drugs in our investigation seemed to influence only total flavonoid content. Total flavonoids and total phenolics content remained the same during 18 months of storage.

Key words: *Tanacetum parthenium; Asteraceae; parthenolide; total flavonoid content; total phenolic content; herbal products quality*

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1. INTRODUCTION

Feverfew (*Tanacetum parthenium* (L.) Schulz Bip., Asteraceae) is a medicinal plant with a long tradition in European folk medicine used in the treatment of fevers, migraine headaches, rheumatoid arthritis, stomach aches, toothaches, insect bites, infertility, problems with menstruation and labor, and also for psoriasis, allergies, asthma, tinnitus, dizziness, nausea, and vomiting (ABC, 2003; Pareek et al., 2011). Nowadays, it is widely used in prophylaxis of migraine headaches available in the market either as fresh, freeze-dried, or dried herb, or in the form of phytopreparations, such as liquid extracts, tablets or more commonly - capsules (Pareek et al., 2011). Stated usage of feverfew in the form of its powdered aerial parts has been approved by the European Medicines Agency (EMA) based upon its long-standing use (EMA, 2010). Usage of feverfew drugs and/or its preparations in migraine headaches has been confirmed in several pharmacological studies and clinical trials. In addition to this, several biological properties, such as anti-inflammatory, chemotherapeutic and anticancer activity, effects on vascular smooth muscle and platelets, as well as inhibition of histamine release have been shown for feverfew extracts and/or its isolated compounds (Pareek et al., 2011). Most of the stated biological activities of feverfew are attributed to sesquiterpene lactones, among which parthenolide is considered the most active chemical constituent. Other potentially active constituents are flavonoid glycosides and pinenes. Also, many components are identified in the volatile oil which is responsible for the strong and bitter odor characteristic for the feverfew plant. Aside being considered the most important biologically active principle of feverfew, parthenolide is widely used as a marker for standardization of its extracts and quality control of finished products, as a prerequisite for the development and production of phytopreparations in accordance with the requests of the

Table 1. Parthenolide content in different organs of the feverfew (*Tanacetum parthenium*) during storage in two consecutive years

	Months	Plant part					Omnipharm extract[a]
		folium	*flos*	*herba*	*herba* [> 0.3 mm]	*herba* [< 0.3 mm]	
		[mg/g]	[mg/g]	[mg/g]	[mg/g]	[mg/g]	[mg/g]
Year 2015	1st	3.95±0.22	15.78±0.51	5.57±0.30	-	-	-
	6th	3.81±0.21	12.83±0.44	3.76±0.30	-	-	-
	18th	2.39±0.22	11.55±0.60	3.65±0.31	-	-	-
Year 2016	1st	6.46±0.24	15.84±0.53	9.04±0.30	-	-	-
	6th	6.22±0.22	14.30±0.50	7.24±0.32	6.58±0.12	7.00±0.08	11.66±0.31
	18th	3.10±0.20	14.11±0.65	4.39±0.29	3.52±0.12	3.60±0.10	10.64±0.33

[a] Commercial extract with standardized parthenolide content (1.1%), according to manufacturer's claims

appropriate regulatory authorities (Kopelman et al., 2008). To this end, the development of adequate chemical methods for identification and quantification of active/analytical markers is an inevitable step in the development and quality assurance of contemporary plant-based preparations not only in the final formulations but also semi-products and herbal raw materials. In other words, for the production of high-quality phytopreparations, herbal drug (substance) of defined quality is imperative. In this context, the aim of the current study was to investigate parthenolide content in various herbal drugs of *Tanacetum parthenium* (L.) Schulz Bip., Asteraceae i.e. flowers, leaves and aerial parts, bearing in mind that the content of this sesquiterpene lactone (not less than 0.20 percent) is defined as the quality criteria for the feverfew herbal drug (dried, whole or fragmented aerial parts) in the European Pharmacopoeia (Ph.Eur.9.0., 2017).

Therefore, parthenolide content in our study was monitored during the storage period of 18 months. In addition, as the phenolic constituents are generally accepted as actives with significant biological properties, total flavonoid (TF) and total phenolic compounds (TP) content were monitored in the investigated samples, as well. Also, in order to evaluate the changes in parthenolide and TF/TP content as a function of particle size, two additional samples (one being >0.3 mm and the other being <0.3 mm in diameter) were prepared from aerial parts of the plants harvested in 2016, taking into consideration that those samples were superior in quality regarding the monitored parameters in comparison to the ones harvested during 2015.

The aim of our investigation was to get more insight into the variation in the percentage of parthenolide content in various parts of the individual plant. This is important in the light of standardization and quality of feverfew supplements.

2. MATERIALS AND METHODS

2.1. Plant material

Plant material was obtained from the Institute for Medicinal Plant Research "Dr. Josif Pancic" (Serbia) and the voucher specimen has been stored at Institute's herbarium, No. TPA15/06, harvest 2015, and No. TPA16/06, harvest 2016. Extract of *Tanacetum parthenium* (L.) Schulz Bip., Asteraceae was purchased from Omnipharm, France.

2.2. Extraction procedure

The extraction procedure of the plant material was conducted according to the procedure described in the European Pharmacopoeia (Ph.Eur.7.0., 2010). One gram of powdered drug was heated with 40 mL of methanol in a water-bath at 60 °C for 10 min. After cooling the mixture was rinsed with 15 mL of methanol and filtered. The procedure was repeated with the residual part. Collected filtrates and rinsings were evaporated to dryness under reduced pressure. The residual part was taken up with the methanol and diluted to 20 mL with the same solvent. After filtration diluted extract was further used for chemical analysis.

2.3. Determination of total phenolic compounds

Total phenolic (TP) content in the samples was determined spectrophotometrically using the Folin-Ciocalteu method (Waterman and Mole, 1994). Two hundred microliters of diluted extracts were added to 1 mL of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 800 µL of sodium carbonate (75 g/L) was added. The absorbance was measured after 2 h of incubation at room temperature, at 765 nm. Gallic acid (0–100 mg/L) was used for calibration of a standard curve. The results were expressed as milligrams of gallic acid equivalents per gram of dry weight of sample (mg GAE/g DW). All experiments were repeated three times.

2.4. Determination of total flavonoids

The total flavonoids content (TF) in tested samples was determined using a modified colorimetric assay previously described by Loizzo et al. (2012). Properly diluted extract (200 µL) was mixed with 60% ethanol (800 µL) and 5% sodium nitrite solution (60 µL). After 5 min, 10% aluminum chloride solution (600 µL) was added and after 6 min 1 M sodium hydroxide solution (400 µL), and the mixture was adjusted to 2 mL with deionized water. The absorbance was measured at 510 nm against the blank (all reagents except the extract). Catechin monohydrate was used to calculate the calibration curve (0.05–0.3 g/L), and results were expressed as milligrams of catechin equivalents per liter of extract (mg CE/L). All experiments were repeated three times.

2.5. HPLC analysis

Analyses were carried out on Agilent 1200 RR HPLC instrument (Agilent, Waldbronn, Germany), with DAD detector, on a reverse-phase LiChrospher (Agilent) analytical column (250 mm × 4 mm i.d.; 5 µm particle size). Isocratic elution was applied and mixture of acetonitrile and water (55:45, v/v) was used as a mobile phase. Stop time of the analysis was 10 min. Detection wavelength was set at 210 nm, and the flow rate was 1 mL/min. The injection volume was 10 µL and the column temperature was maintained at 25 °C. The identification of parthenolide was based on comparison of retention time and UV spectra with those from the authentic standard while its amount was calculated using calibration curve. The results are presented as milligrams per gram of dry weight. All experiments were repeated three times.

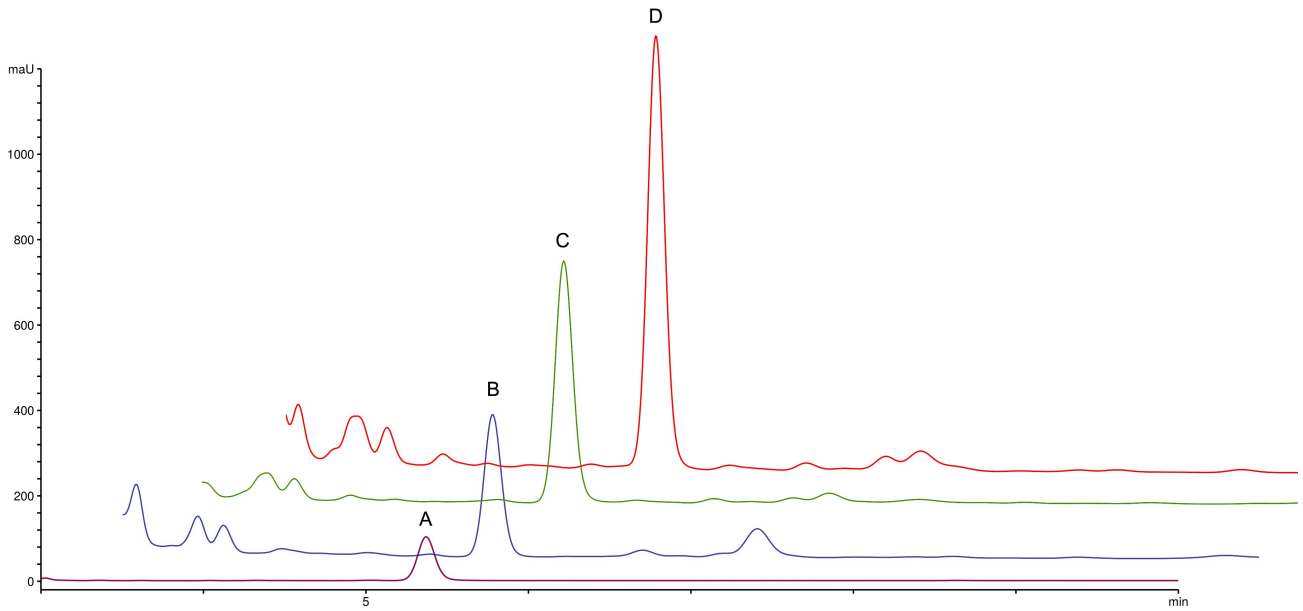

Fig. 1. HPLC chromatograms of investigated samples and standard recorded at λ = 210 nm: A) parthenolide standard; B) *Tanaceti parthenii folium* - 2015 (sample diluted 2 times before injection); C) *Tanaceti parthenii flos* – 2015 (sample diluted 3 times before injection); D) *Tanaceti parthenii herba* - 2015 (non-diluted sample)

3. RESULTS AND DISCUSSION

Herbal product's quality is dependent on the content of biologically active constituents present in the herb. The sesquiterpene lactone parthenolide is identified as one of the compounds responsible for some of feverfew's beneficial effects (Benassi-Zanqueta et al., 2018). Beside maceration, several other techniques have been used for its extraction such as Soxhlet and bottle stirring (Zhou et al., 1999), high-pressure extraction with CO_2 (Čretnik et al., 2005; Kery et al., 1999) and extraction under vacuum (Marete et al., 2009). Also, various extraction solvents were applied. They usually included chloroform (Marchand et al., 1983), petroleum ether (Awang et al., 1991), acetone (Heptinstall et al., 1992) and acetonitrile (Abourashed et al., 2000). Wu et al. (2006) extracted parthenolide, luteolin, apigenin from feverfew in 80% alcohol, while Marete et al. (2009) applied distilled water heated at various temperatures. Physiochemical methods were used for parthenolide quantification in several commercial products with *T. parthenium*. The results varied significantly and, in some products, parthenolide was not detected (Ghafari et al., 2010).

According to our results (Table 1, Figure 1) there is a wide variation in the amount of parthenolide in different parts of the herb. The highest content was recorded in flower samples (15.78 mg/g), while the lowest level was detected in leaf samples (3.95 mg/g). This is in accordance with previous studies. Heptinstall et al. (1992) showed that the highest content of parthenolide was found in flower heads (138 mg/100 g of raw material), followed by leaves (95 mg/100 g of raw material), and with only 80 mg/100 g raw material in stalks and 10 mg/100 raw material in roots. Similarly, Majdi et al. (2011) exhibited the highest amount of parthenolide in flower heads, while no parthenolide was detected in feverfew roots. The same group of authors showed that the parthenolide content varied significantly between the two components of the flower. The highest amount was found in the disc florets when compared with the ray floret and this fact corresponded to a higher density of glandular trichome on the disc florets.

During the period of 18 months, while the investigated samples were stored and kept under storage conditions mimicking the usual manner of keeping the plant raw material (leaves, flowers and herbs) and dry extracts, adopting the managing quality to prevent degradation, contamination, and cross-contamination, in appropriate containers, it could be observed that in the extract commercially available at the market the parthenolide content decreased less than 0.1%, in contrast to the investigated *T. parthenii* drugs (leaves, flowers and aerial parts), in which a greater percentage of parthenolide content decrease was noticed (Table 1).

Regarding leaves, Végh et al. (2014) demonstrated that the highest content of parthenolide was found in leaves collected before flowering compared to the leaves collected during the flowering period. That implies that with the beginning of the flowering period a decrease of parthenolide contents in the leaves arises. Parthenolide level can also decline during storage (Heptinstall et al., 1992). This has also been confirmed in our results (Table 1). The particle size of powdered samples is a significant factor that could affect the content of bioactive constituents. Parthenolide could be located in trichomes or bonded densely in other tissues. These various locations can potentially demonstrate the significance of obtaining finely grounded samples for maximum extraction yields (Fonseca et al., 2006). According to this group of authors parthenolide extraction from unsieved samples was higher compared to extraction from 1000-500 µm particle size and lower compared to parthenolide content obtained from samples of <500 µm particle size. From our results presented in Table 1, it can be seen that slightly greater content of parthenolide was obtained in herb samples with particle size lower than 0.3 mm in comparison to the higher one.

European Pharmacopoeia (Ph.Eur.7.0., 2010) requires minimum 0.2% of parthenolide content for *T. parthenium* aerial part. According to our results, for all of the investigated samples this condition was met. Even though the flower heads might contain more than 1.0% of parthenolide, which is in accordance to our results as well (Table 1) it was not convenient to establish *T. parthenii* flowers as an independent phytotherapeutic drug, since the yield of flower heads was fairly small (about 20% related to the whole drug) (Hendricks et al., 1997).

Table 2. Total flavonoid content in different organs of the feverfew (*Tanacetum parthenium*) during storage in two consecutive years

	Months	Plant part					Omnipharm extract[a]
		folium	*flos*	*herba*	*herba* [> 0.3 mm]	*herba* [< 0.3 mm]	
		[mgCatE/g]	[mgCatE/g]	[mgCatE/g]	[mgCatE/g]	[mgCatE/g]	[mgCatE/g]
Year 2015	1st	863.6±38.1	315.0±25.1	292.7±23.1	-	-	-
	6th	855.7±36.2	315.0±25.1	290.7±25.1	-	-	-
	18th	855.4±38.2	313.9±22.9	290.7±25.1	-	-	-
Year 2016	1st	697.6±32.4	340.2±25.8	336.9±25.1	436.3±33.2	665.7±35.6	670.9±36.1
	6th	690.6±31.5	340.0±25.8	332.5±29.1	430.7±37.1	662.3±35.1	667.2±35.9
	18th	690.6±38.1	339.7±22.7	332.1±29.1	430.2±35.9	661.9±35.1	666.5±35.8

[a] Commercial extract with standardized parthenolide content (1.1%), according to manufacturer's claims

Table 3. Total phenolis content in different organs of the feverfew (*Tanacetum parthenium*) during storage in two consecutive years

	Months	Plant part					Omnipharm extract[a]
		folium	*flos*	*herba*	*herba* [> 0.3 mm]	*herba* [< 0.3 mm]	
		[mgGAE/g]	[mgGAE/g]	[mgGAE/g]	[mgGAE/g]	[mgGAE/g]	[mgGAE/g]
Year 2015	1st	14.9±2.0	12.5±1.1	11.5±1.1	-	-	-
	6th	14.7±1.3	12.1±1.5	11.2±0.9	-	-	-
	18th	14.4±1.3	11.9±1.5	11.2±1.3	-	-	-
Year 2016	1st	17.7±1.0	10.9±0.9	15.2±2.3	18.3±2.2	15.9±2.1	55.2±2.5
	6th	17.6±1.5	10.9±1.1	15.2±2.3	18.1±2.5	15.5±1.9	55.1±2.1
	18th	17.5±1.5	10.7±1.3	15.0±2.3	18.0±2.5	15.5±2.1	55.0±2.5

[a] Commercial extract with standardized parthenolide content (1.1%), according to manufacturer's claims

In addition, taking into account that the presence of flavonoids and phenolic compounds might significantly affect the overall activity of the phytopreparations, we monitored their amount in the tested samples during the investigated period of time. It was noticed that their content was lower in comparison to data reported in the literature (Wu et al., 2006). Our results revealed that TF and TP content did not decrease significantly over the 18-months period (Tables 2 and 3).

CONCLUSION

The results of our study revealed that the content of parthenolide decreased during the investigated period (from 3.95 to 2.39, from 15.78 to 12.83 and from 5.57 to 3.65 mg parthenolide/g in leaves, flowers and aerial part, respectively, obtained during the harvest 2015, and from 6.46 to 3.10, from 15.84 to 14.11, from 9.04 to 4.39 mg parthenolide/g of the samples (leaves, flowers and aerial part, respectively), obtained during the harvest 2016). In contrast, parthenolide content in the commercially available extract did not change during the time of the investigation. Different particle size of the tested drugs in our investigation seemed to influence only total flavonoids content. Total flavonoids and total phenolics content remained the same during 18 months of storage.

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