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COMPARATIVE ANALYSIS OF PARTHENOLIDE CONTENT IN *Tanacetum larvatum,* AN ENDEMIC SPECIES OF MONTENEGRO, COLLECTED FROM THREE DIFFERENT LOCATIONS

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Plants from the genus *Tanacetum* L. (Asteraceae) have been used in traditional medicine from ancient times, of which *Tanacetum parthenium* (L.) Shultz-Bip. (feverfew) has been known as a remedy for the treatment of various diseases, including migraine, arthritis, fever, vertigo, menstrual disorders, stomach-ache, and psoriasis [1]. Additionally, *T. vulgare* L. (common tansy) and *T. microphyllum* DC. are useful in the treatment of various inflammatory disorders [2, 3]. Recent investigation of *T. larvatum* extract suggested the application of this species as an alternative or supplementary herbal remedy for the treatment of inflammatory diseases due to its anti-inflammatory and anti-ulcer activities [4]. The secondary metabolites that mediate these pharmacological effects are mainly biologically active sesquiterpene lactones, such as parthenolide and hydroxyachillin. Parthenolide, found in significant amounts in feverfew, has been indirectly linked to the anti-migraine action of feverfew preparations [5], as well as to anti-tumor and anti-inflammatory properties [6, 7]. The mode of parthenolide activity comprises the inhibition of prostaglandin production [8] and 5-hydroxytryptamine secretion [9] as mediators of inflammation. Besides, parthenolide was shown to suppress phorbol-induced mouse ear and carrageenan-induced rat paw edema, inhibiting the platelet activation [10, 11] and impairing the activity of the transcriptional factor NF-kB [12, 13]. Herrera et al. reported a strong intracellular antioxidant activity of this sesquiterpene lactone in hippocampal HT22 cells [14]. As Miglietta et al. showed that parthenolide exerted *in vitro* stimulatory activity on tubulin assembly, the tubulin/microtubule system may represent a novel molecular target to be utilized in developing new combinational anticancer strategies [15]. Recent investigation, confirmed its gastric anti-ulcer properties due to its ability to restore the reduction of sulfhydryl groups within the gastric mucosa and to increase mucosal PGE_2 level [3, 16].

Feverfew from Serbia, as previously reported, does not contain parthenolide [17]. Because of the assumed importance of parthenolide content for pharmacological activities, supported by the studies already performed, we investigated the variation of parthenolide content in *T. larvatum* (Gris.) Kanitz. from Montenegro [18, 19], during a five-year period. Collected materials from three different locations were subjected to quantitative ^{1}H NMR and HPLC analysis in order to get more insight whether the parthenolide content in the *T. larvatum* aerial parts is a stable property during five years period of investigation. We also measured the reduction of DPPH absorption by investigated *T. larvatum* extracts to indicate the capacity of the extracts to scavenge free radicals, independent of any enzymatic activity.

Quantitative ^{1}H NMR and HPLC analysis revealed the parthenolide content (Table 1) in the aerial parts of *T. larvatum*, collected during the phase of flowering at three natural habitats in Montenegro, Komovi Mountain (Sample *a*), Prokletije Mountain (Samples *b*), and Sinjajevina Mountain (Sample *c*), in the period of 2001–2005. Concerning that the amount of parthenolide might decrease during storage, the quantitative analysis was performed immediately after collection and drying of the plant material. The parthenolide level ranged from 0.40 to 1.93%, and the presented results were in accordance with reported data [20]. Although variations in the percentage were registered, the parthenolide content in all samples was comparable to that found in feverfew. Moreover, it is evident that the parthenolide amount varied during the investigated period, that in sample *c* being the most consistent.

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TABLE 1. The Parthenolide Content in *T. larvatum*, Collected at Different Locations in Montenegro Determined by ^{1}H NMR and HPLC (in parenthensis)

Sampling year	Parthenolide, %[a]		
	sample *a*	sample *b*	sample *c*
2001	1.10±0.03 (1.13±0.07)	0.74±0.21 (0.70±0.04)	0.79±0.02 (0.71±0.06)
2002	1.93±0.01 (2.00±0.1)	1.12±0.01 (1.02±0.08)	0.77±0 (0.71±0.07)
2003	0.40±0.10 (0.49±0)	0.98±0 (0.91±0.02)	0.80±0.02 (0.77±0.01)
2004	0.79±0.01 (0.81±0.01)	0.89±0.03 (0.81±0.01)	0.76±0.01 (0.71±0.01)
2005	0.59±0.02 (0.53±0.03)	1.35±0.02 (1.41±0.09)	0.91±0.01 (0.89±0.05)

[a]Parthenolide content represents the mean value ±SD of three measurements.

TABLE 2. EC_{50} values in DPPH assay of investigated *T. larvatum* extracts

Sample	EC_{50} (μg/mL)[a,b]	Sample	EC_{50} (μg/mL)[a,b]
Sample *a*	102.5±6.1	Trolox	5.9±0.8
Sample *b*	165.9±10.9	BHT	6.0±0.2
Sample *c*	131.0±7.2		

[a]Sigmoidal fit. [b]EC_{50} represents the mean value ±SD of five measurements.

There was generally good agreement between the values for parthenolide content derived from both ^{1}H NMR and HPLC assay procedure (Table 1). The extracts possessed strong DPPH free radical-scavenging activity, with the determined EC_{50} values presented in Table 2. Sample *a* was the most active, with a determined EC_{50} value of 102.5 μg/mL. The observed antioxidant properties are comparable to those of the feverfew alcoholic extract [21].

Bearing in the mind that *T. parthenium* native to Serbia does not contain parthenolide, of chemotaxonomic importance are the intriguing results concerning the parthenolide content and antioxidant activity of *T. larvatum*, which were comparable, even higher, than those of *T. parthenium.* The present data support further investigation of this species as a new commercial source of parthenolide or potential herbal remedy with antimigraine, anti-inflammatory, and gastroprotective effects.

Plant Material. Aerial parts were collected in July and August during the period 2001–2005 on three locations in Montenegro. Plant material was identified by Prof. Vladimir Stevanovic from the Institute of Botany, University of Belgrade. Voucher specimens have been deposited at the Botanical Garden, Faculty of Biology, Belgrade, No. 1404, No. 1405 and No. 1406.

Extraction. The samples of fresh plants were air dried at room temperature. The extracts (MeOH–EtO_2–petroleum ether, 1:1:1; yield: 8.4, 7.5 and 8.2%, w/w, for Samples *a*, *b*, and *c*, respectively) of ground air-dried aerial parts of *T. larvatum* were prepared according to the usual procedure for isolation of sesquiterpene lactones [22].

Isolation of Parthenolide. *T. larvatum* was collected in July 2001 at Komovi Mountain, at the same place as the studied sample, for the isolation of parthenolide as the main constituent in the extract. The extract (MeOH–Et_2O–petroleum ether, 1:1:1) of ground air-dried aerial parts of the crop (270 g), prepared by the procedure already mentioned [22], was subjected to a combination of dry-column flash chromatography, MPLC, and silica gel CC to yield the germacranolide parthenolide (1.35 mg). The structure of the isolated compound was identified by ^{1}H NMR spectra and compared to literature [23, 24].

Procedure for ^{1}H NMR and HPLC Analysis. The crude extract was subjected to quantitative ^{1}H NMR, using BHT as internal standard, as already reported [20]. For HPLC analysis the samples were prepared in the same way as for ^{1}H NMR analysis and dissolved at a concentration of 10 mg/mL in MeOH (HPLC grade). The parthenolide content was determined using the procedure described previously [25].

Procedure for DPPH Scavenging Activity. The DPPH scavenging assay was carried out according to the procedure described by Blois, with some modification [26]. The inhibition percentage was calculated using the following equation: $I = [(A_c-A_s)/A_c] \times 100$, where I is the inhibition percentage, A_c is the absorbance of the negative control, and A_s is the

absorbance of the samples. Synthetic antioxidants, Trolox and BHT, were used as positive controls. The inhibition percentage was plotted against concentration of the samples, and the EC_{50} values, determined by linear regression analysis, are presented as mean ± standard deviation of five determinations.

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