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Short Communication

PHYTOCHEMICAL ANALYSIS OF SECONDARY METABOLITES OF *SATUREJA HORTENSIS* L.

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ABSTRACT

Objective: The present study was designated to analyse the essential oils and tannins as important secondary metabolites of the aerial part of *Satureja hortensis*.

Methods: The chemical composition of *S. hortensis* herb was investigated using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). The essential oil was obtained by hydrodistillation method.

Results: The essential oil yield in the aerial part of *S. hortensis* was 1.61%. GC-MS analysis of the essential oils identified the presence of 29 components. Carvacrol (76.16%), as the main component of essential oils, belongs to the group of aromatic compounds. Eight tannin components identified by HPLC and epigallocatechin (130.91×10^{-2}%) are prevalent among them.

Conclusion: *S. hortensis* was found to possess considerable amount of phytoconstituents such as essential oils and tannins. The results of this research will help to study pharmacological properties of the investigated plant and to prevent possible adulteration with other plants.

Keywords: *Satureja hortensis*, Essential Oil, Tannins, HPLC, GC-MS.

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Plants synthesise a vast range of organic compounds classified into primary and secondary metabolites [1]. Primary metabolites are involved in the growth and development, respiration and photosynthesis, hormone and protein synthesis; secondary metabolites play an important role in protecting the plant body from external biotic impacts and damaging as signalling components (to attract animals during pollination or distribution of seeds and fruits) [2]. Accumulation of various metabolites depends on numerous factors, particularly plant chemotype and growth conditions (rain, soil, temperature, etc.) [3-6].

Summer savory (*Satureja hortensis* L., *Lamiaceae*) is an annual aromatic and ornamental plant. It is used in folk medicine as antiseptic, antimicrobial, and antispasmodic agent for the treatment of the respiratory tract, skin and gastrointestinal system disorders [7]. This non-officinal medicinal plant is not included in European Pharmacopoeia [8] and not mentioned in modern reviews [9, 10].

Comprehensive research of insufficiently studied medicinal plants includes their phytochemical, pharmacological, morphological and anatomical analyses [9–11]. The species of *Lamiaceae* Family are an abundant source of such important groups of secondary metabolites as essential oils and phenolic compounds [1, 4, 5]. In scientific literature there is no information about determination of tannins composition in *S. hortensis* with HPLC method and the GC-MS analysis of essential oils of *S. hortensis,* therefore, it is of great current interest because the plant can grow in different climate conditions [5, 6].

The present study was aimed to make the qualitative and quantitative analyses of essential oil and tannins in the aerial part of *S. hortensis* was harvested at the full-flowering stage in 2013 and 2014 respectively from the experimental plots in Ternopil region (Ukraine, 49.5535 °N, 25.5948 °E). The climate of this area is moderate continental [12]. The herb was dried at 30-35 °C, then reduced to the fine powder and placed in tightly closed containers. Plants were grown from the seeds obtained from the collection of M. Hryshko National Botanical Garden in Ukraine. Herbarium specimens of the plants (Lsh-112) have been included in the herbarium of Pharmacognosy and Medical Botany Department of I. Horbachevsky Ternopil State Medical University.

The essential oil was isolated from the powdered *S. hortensis* (10.0 g) by distillation for 3 h using a Clevenger apparatus according to the European Pharmacopoeia [8]. The essential oil was collected from the nozzle of the condenser and dried under anhydrous sodium sulphate. It was stored at 4-6 °C for further identification.

Identification of essential oil components was done using GC-MS (Agilent Technologies 6890/5975C chromatograph) [13]. A DB-5 capillary column with a length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 μm was used for gas chromatographic separation. Sample injection was performed in the splitless regime, and volume of extract injected was 1 μL. Helium with a purity of 99.999% was used as carrier gas at a constant flow of 1.2 ml/min. The procedures were carried out in the programmed mode with temperature programming from 50-320 °C at a rate of 4 °C/min. The mass spectrometer was operated in the electron impact ionisation mode (70 eV). Identification of compounds was done with using the library of mass spectra of the reference compounds (National Institute of Standards and Technology, USA).

Investigation of tannins was performed by HPLC (chromatograph Agilent 1200 3 D LC System Technologies, USA) [14]. It is equipped with diode array detector (G1315C), PC software Agilent ChemStation (G2215BA), autosampler (G1329A), thermostat of column (G133OB), four-channel pump of low pressure gradient (G13111A), vacuum degasser (G1322A), a Discovery C_{18} column (250 x 4.6 mm, Supelco, № 505129) with the precolumn (20 mm, grain size 5 μm). Solution A (0.1% trifluoroacetic acid solution, 5% acetonitrile solution) and solution B (0.1% trifluoroacetic acid and acetonitrile) were used as mobile phase. The column thermostat temperature was 25 °C, the volume of injected samples: 5-20 μm, flow rate: 0.7 ml/min, chromatography time: 40 min, scan time: 0.6 seconds, detection range: 190-400 nm, the wavelength: 280 nm.

Extraction procedure for HPLC was done as follows: 1.0 g of grinded plant material was placed in 100 ml flask with 50 ml of distilled water added. The flask was placed in water bath for 30 min. The obtained solution was carefully filtered through a membrane filter (0.45 μm pore size) and placed into a vial. Solutions of sample

substances standards were prepared at the concentration of 50-200 mg/l by five-stage calibration in manual mode.

All reagents for GC-MS and HPLC received from Sigma-Aldrich, USA was of analytical grade (>95 % purity).

The essential oil yield obtained from *S. hortensis* was 1.61%. The obtained essential oil is a yellowish-orange liquid with fragrance and slight irritating smell**;** its taste is bitter and spicy.

The result of GC-MS analysis of the essential oil is presented in table 1 and fig. 1. A total of 29 compounds were isolated and 28 of them were identified. Aromatic alcohol carvacrol (76.16%) dominates in the investigated essential oil. The essential oil also accumulates the significant quantity of γ-terpinene (10.16%). The other components comprise less than 3%.

By HPLC it was identified a number of tannin components (fig. 2, 3). The presence of such compounds as epigallocatechin ($130.91x10^{-2}$%), gallocatechin ($73.87x10^{-2}$%), epicatechin ($47.22x10^{-2}$%), catechin ($35.18x10^{-2}$%), epicatechin gallate ($31.13x10^{-2}$%), catechin gallate ($16.55x10^{-2}$%), gallic acid ($2.17x10^{-2}$%), and ellagic acid ($1.11x10^{-2}$%) was found out. Most of them were detected in *S. hortensis* for the first time.

Among the secondary metabolite compounds identified in *S. hortensis* the essential oil was a significant one. It is known that essential oils are very complex natural mixtures which contain about 20 or more components at quite different concentrations [2, 5, 9]. Among the compounds identified in essential oils of *S. hortensis*, the carvacrol is a significant one. This aromatic alcohol has low toxicity and antiseptic properties [15–18].

The second quantitative component of essential oil is γ-terpinene which is used in cosmetics and food industries as flavoring chemical. The other components (β-caryophyllene, β-bisabolen etc.) have neuroprotective, antinociceptive and anti-inflammatory properties [9, 19-23].

Table 1: Chemical compounds identified in essential oils of *S. hortensis* herb through GC-MS analysis

S. No.	RT	Component	Content %
1.	6.32	myrcene	0.14
2.	6.75	1-octen-3-ol	1.00
3.	6.92	3-octanol	0.21
4.	7.24	α-terpinene	0.29
5.	7.54	limonene	0.06
6.	8.29	trans ocimene	0.10
7.	8.36	p-cymene	0.81
8.	8.89	γ-terpinene	10.16
9.	9.51	trans sabinene hydrate	0.95
10.	10.51	linalool	0.37
11.	10.85	cis-sabinenhydrate	0.45
13.	13.86	terpinen-4-ol	0.50
14.	15.15	α-terpineol	0.17
15.	16.35	methyl carvacrol	0.12
16.	17.62	neral	0.39
17.	18.79	thymol	0.60
18.	19.62	carvacrol	76.16
19.	19.91	β-caryophyllene	2.67
20.	20.07	ascaridole	0.35
21.	20.27	aromadendren	0.13
22.	20.89	humulene	0.29
23.	21.55	germakren D	0.21
24.	21.73	β-bisabolen	2.32
25.	22.04	bicyclogermacrene	0.56
26.	22.55	cis-α-bisabolen	0.17
27.	24.28	spatulenol	0.22
28.	24.37	caryophyllene	0.12
29.	32.81	not identified	0.50

It was established that the predominant components of tannins in the aerial part of *S. hortensis* are derivatives of flavan-3-ol (condensed tannins). Tannins have the unique property of forming stable complexes with proteins of membranes and have astringent and anti-inflammatory effects. They can coagulate the mucosal tissues by creating a protective layer that soothes irritation and pain on the skin [24]. Their water-soluble properties allow extracting them easily for using in pharmaceutical industry [25].

The mechanisms of phenolic metabolites actions are also intended to their antioxidant activity and ability to neutralise the lipid free radicals; they can prevent the damaging influence of hydrogen peroxide [26, 27]. Catechins and other polyphenols are effective absorbers of reactive oxygen species; they are extensively metabolised in the body, which underlines their important role in the manifestation of antioxidant, anti-inflammatory and anticarcinogenic activity [28].

Many of identified components of essential oils and tannins have similar pharmacological action and could be to a great extent responsible for the anti-inflammatory, antioxidant, neuroprotective and antimicrobial activity.

The present study revealed that the aerial part of *S. hortensis* accumulates a considerable amount of constituents such as essential oils and tannins. A lot of components were identified in the investigated plant for the first time. The obtained results will help in the phytochemical research of *S. hortensis* for standardisation of the herbal powdered drug and preventing possible adulteration. The identified compounds are of interest for further pharmacological studies too.

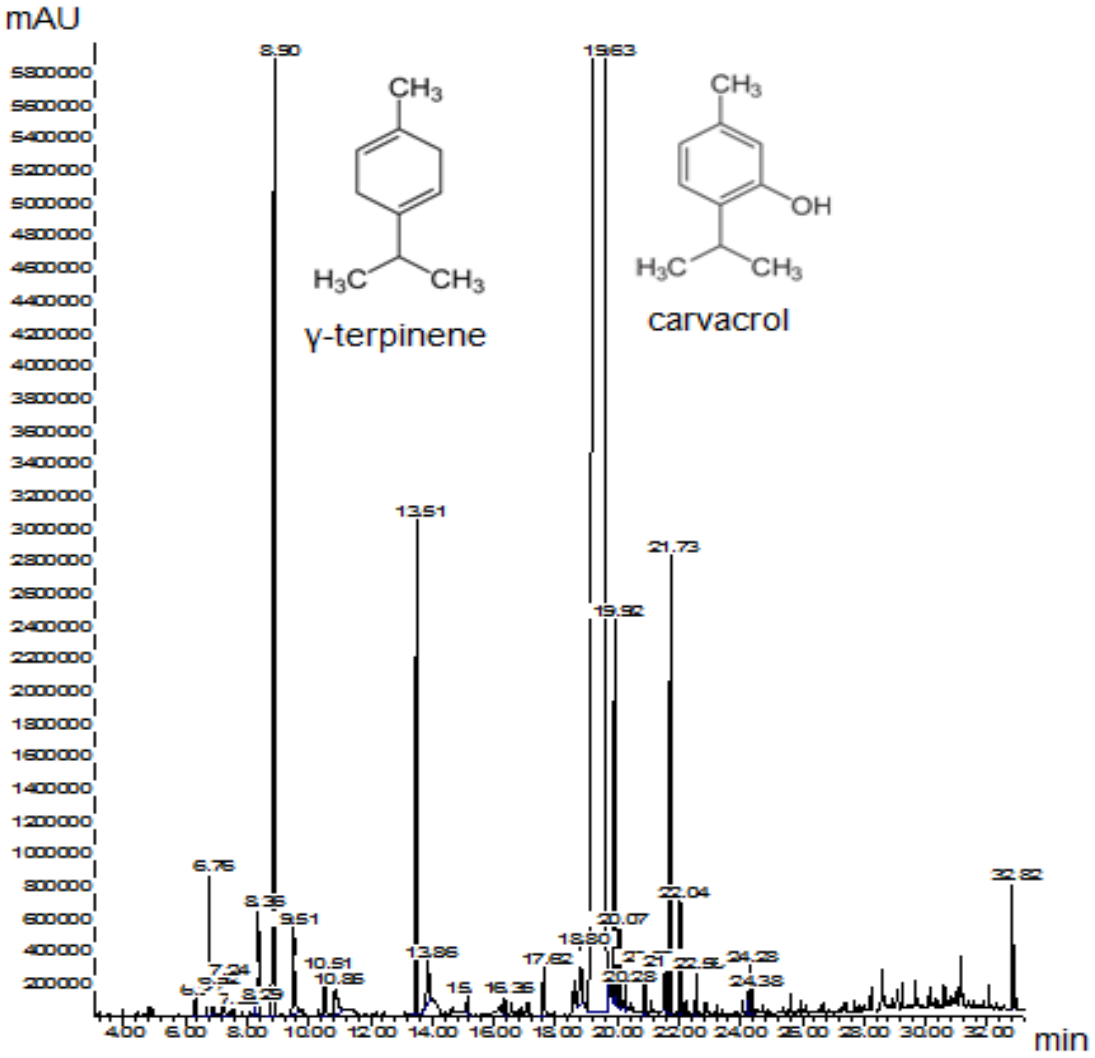

Fig. 1: GC-MS Chromatogram of essential oil of *S. hortensis* herb

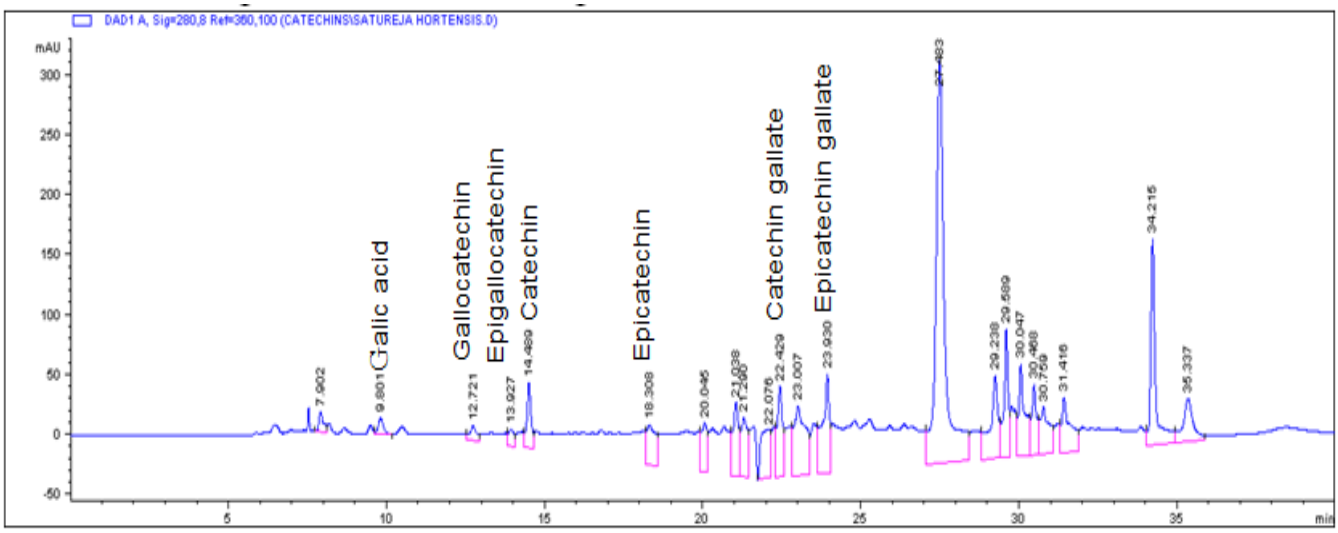

Fig. 2: HPLC chromatogram of water extract of *S. hortensis* herb

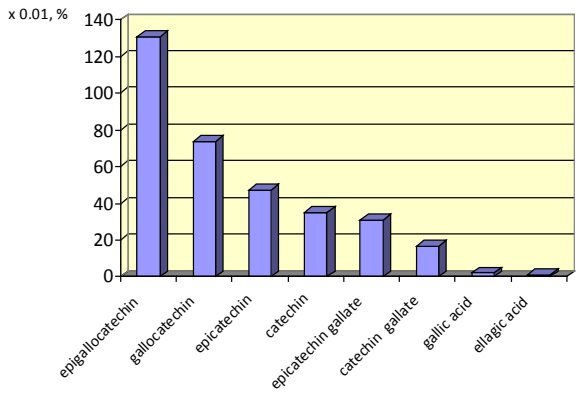

Fig. 3: Comparative content of tannins in the water extract of *S. hortensis* herb

CONFLICT OF INTERESTS

Declared none

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