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CHROMATOGRAPHIC ANALYSIS OF POLYPHENOLS IN THE *SATUREJA HORTENSIS* L. (*LAMIACEAE* MARTINOV) HERB

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Abstract

Background and objectives: The health promoting properties of aromatic medicinal plants are of great importance for the pharmaceutical and food industries. **Methods**: the chromatographic analysis of phenolic compounds in the summer savory (*Satureja hortensis* L.) herb cultivated in western Ukraine was carried out using high-performance thin layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) methods. **Results**: The specific polyphenolic profiles of the *S. hortensis* herb was evidenced by this study. The rosmarinic acid (RA), caffeic acid (CA) and chlorogenic acid (ChA) were identified by the HPTLC method. Such polyphenols as RA, CA, ChA, ferulic acid, neochlorogenic acid, apigenin, luteolin, catechin, quercetin, rutin, hyperoside, acacetin-7-*O*-glucoside, apigenin-7-*O*-glucoside, and luteolin-7-*O*-glucoside were detected and quantified using HPLC. The significant amount of RA (21.42 mg/g) followed by the flavone derivatives and CA was revealed. Found predominant phenolic compounds possess the proven therapeutic properties. **Conclusion**: The obtained results can be useful for establishing the quality parameters of the investigated herb. We expect our results open new directions for designing valuable drugs and functional food additives.

Keywords: *summer savory, HPTLC, HPLC, polyphenols, rosmarinic acid*

Introduction

Plant-based medicines have been widely used in the prevention and treatment of many diseases since ancient time [1, 2]. Their multiple components provide the synergistic mode of actions of the plant extracts demonstrating stronger beneficial effects than alone ones [3, 4]. Bioactive compounds of plants are preferably secondary metabolites possessing valuable therapeutic properties [5].

The health promoting properties of medicinal plants are of great importance for the pharmaceutical industry [2]. The significantly growing interest of the food industry for the phytoconstituents of aromatic plants has been increasing due to harmful properties of synthetic antiseptics and antioxidants [6, 7].

The representatives of the *Satureja* L. genus (*Lamiaceae* Martinov family) have long history of use as the medicinal plants, spices and herbal teas. According to [8], this genus comprises 52 species and a number of subspecies of herbaceous or semi-woody aromatic plants common to Asian and the Mediterranean countries [9, 10]. The most widely cultivated species in Europe are winter savory (*S. montana*) and summer savory (*S. hortensis*) [9, 11, 12]. Last species is more favorable for cultivation in temperate climate [9].

S. hortensis is an annual herb up to 40 cm tall with thin rounded-quadrangular stems and linear-lanceolate leaves. Violet-purple flowers are collected in semicircles in the apical intermittent spike-shaped inflorescences [6, 11]. The aerial part of *S. hortensis* has been used in a folk medicine as bactericidal, antitussive, analgesic, antispasmodic, and antiemetic remedy [9, 13–15]. Various isolates such as essential oils, infusions, extracts, tinctures are obtained from this plants [9]. *In vitro* and clinical studies have confirmed its effectiveness as an astringent for treating stomatitis, bronchitis, gastrointestinal disorders, stomach colic, tumor, etc. [13, 14]. It is also successfully applied as a spice in the food industry due to the antioxidant and antimicrobial properties [6, 9].

Volatile terpenoids and polyphenols have been reported as the main active principles of the *S. hortensis* aboveground organs [15–20]. Triterpene compounds were also reported [21]. As it is known, the variability in the contents of certain bioactive compounds in the plant raw materials depends on its geographical origin, chemotype, climate conditions, phase of vegetation, extraction procedure and can vary quite significantly [22, 23]. Therefore, the phytochemical analysis of plants should be conducted taking into account these features.

The aim of present study was the chromatographic analysis of phenolic compounds in the *S. hortensis* herb cultivated in western Ukraine using two methods: high-performance thin layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) methods.

Methods

Plant material

The herb of *S. hortensis* was harvested from blooming plants under the cultivation in Ternopil region (western Ukraine) and shade dried.

Chromatographic analysis

The HPTLC analysis was performed using Linomat 5 devices (CAMAG, Switzerland) as it described by [19]. Methanol was used for the extraction of phenolic compounds from the powdered herb of *S. hortensis*. 1.0 g of the plant raw material was macerated over a 3 h in 10 mL of methanol using periodical shaking. 1 mL of the obtained extract was filtered and used for the analysis as a test solution. Standards of rosmarinic acid (RA), caffeic acid (CA) and chlorogenic acid (ChA) for HPTLC were purchased from Sigma-Aldrich (Poland). To prepare the standard solutions the standards (1 mg) were dissolved in 4 mL of methanol. The chromatographic separation was conducted on 20 x 10 cm TLC silica gel plates F_{254}. The mobile phase consisting of ethyl acetate – formic acid – water (15:1:1) was chosen for the eluting process.

The detection of phenolic compounds was conducted before and after the post-chromatographic derivatization with 1% $AlCl_3$ under UV light at 366 nm. The investigated compounds were determined using the R_f value and fluorescence due to comparing with standard solutions. All chemicals were of analytical grade.

The HPLC analysis was conducted for or the quantitative evaluation of polyphenols in the 70% ethanol extract of *S. hortensis*. The extraction procedure was conducted with the boiling water bath. Chromatograph Shimadzu HPLC-DAD system with the C18 Phenomenex Luna column (250 mm x 4.6 mm) was applied. The gradient elution was provided by mixing two mobile phases: A (0.1% trifluoroacetic acid in water) and B (0.1% trifluoroacetic acid in acetonitrile) according to [24]. The reference standards of hydroxycinnamic acids (RA, CA, ChA, ferulic acid, neochlorogenic acid) and flavonoids (apigenin, luteolin, apigenin-7-*O*-glucoside, L-7, acacetin-7-*O*-glucoside, catechin, rutin, quercetin, hyperoside) were purchased from Merck (Germany). The UV absorption spectra of the reference standards and test samples were recorded at λ=190–400 nm. All chemicals were of analytical grade.

All the analyzes were carried out at least in five repetitions.

Results and Discussion

The HPTLC method was used for obtaining the 'chromatographic fingerprints' of phenolic compounds in the *S. hortensis* herb. The results are displayed in Figure 1. The chromatograms of test solution showed the most intense light blue fluorescent zone in the upper part corresponding to RA (R_f= 0.75). The weaker light blue zone of caffeic acid was detected at R_f= 0.79. The more intense unidentified zone in a blue fluorescence was found below the RA spots. The lower third of tracks contained zones of yellow fluorescence (after the derivatization) which could be referenced to flavonoids. The red stripes corresponding to chlorophyll were observed directly below the solvent frontline.

The contents of five hydroxycinnamic acids (CA, ChA, RA, ferulic, neochlorogenic) were evaluated using HPLC (Table 1). Regarding flavonoids, four aglycones (quercetin, catechin, apigenin and luteolin) and five glycosides (rutin, hyperoside, acacetin-7-*O*-glucoside, apigenin- 7-*O*-glucoside and luteolin-7-*O*-glucoside) were found. The obtained HPLC chromatogram is displayed in Figure 2.

As can been seen from the results displayed in Table 1, the RA was the main polyphenol of the investigated species. Our findings are comparable to data regarding many other *Lamiaceae* representatives [12, 15, 23, 25, 26]. Thus, the methanol extracts of *S. hortensis* herb contained18.77 mg/g RA as the major compound [19]. Another study [12] reported that *Satureja montana* herb accumulated up to 7.84 mg/g RA. However, when infusion of this herbs was prepared, the content of RA component was much lower (3.64 mg/g).

A water-ethanol extract of *Melissa officinalis* (*Lamiaceae*) leaves gave about 29.70mg/g of this hydroxycinnamic acid [26]. The total phenolic contents calculated as RA equivalent in the leaves and stems of *Melissa officinalis* were in the range34.1–81.9 mg/g [23]. According to Benedec et al. [25], among 6 indigenous *Lamiaceae* species (*Hyssopus officinalis* L., *Melissa officinalis* L., *Ocimum basilicum* L., *Origanum vulgare* L., *Rosmarinus officinalis* L. and *Salvia officinalis* L.) the water-ethanol extract of *Origanum vulgare* contained the largest amount of RA (12.40 mg/g).

Studies carried out by Hadian et al. [27] demonstrated the considerable variation of RA content (from 0.06% to 0.69%) in the methanol extracts of Iranian *hortensis* raw material dependently on the collection place. Alcohol extract of this species collected at budding phase in the Lithuania climatic conditions contains the largest contents of polyphenols where the RA and rutin prevailed [13]. Bros et al. [16] found the dependence of the RA and luteolin amounts in the *S. hortensis* herb on different extraction techniques (reflux, maceration, microwave) and solvents. RA commonly with CA and ChA were also considered as the quality markers of the *Monarda fistulosa* herb [28].

As can be seen from the Table 1, such flavones as apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside dominated among flavonoids in the studied herb. The amounts of catechin, rutin and neochlorogenic acid were remarkably low. It should be noted that Damašius et al. [29] firstly found the phenolic lithospermic acid in the Lithuanian *S. hortensis*.

The significant content of phenolic compounds strongly correlated with the pronounced antioxidant activity was characteristic of the water extract Turkish *S. hortensis* herb comparatively with

ethanol or acetone ones [20].

Found polyphenols as well as many other compounds of the complex plant extracts possess the proven therapeutic potential [30–45]. The ability of polyphenols to stabilize free radicals are due to containing two or more OH-groups associated with the aromatic ring [32]. The RA possessing four hydroxyl groups has the considerable antioxidant, antibacterial, antiviral, immunomodulatory, hepatoprotective and anti-inflammatory properties [31, 34]. Findings of Khan et al. [35] revealed that CA can effectively suppress the oxidative stress. Salehi et al. [37] described the antioxidant, anti-inflammatory, antispasmodic. etc. therapeutic properties of apigenin. The antioxidant potential of luteolin and its derivative luteolin-7-O-glucoside was detected *in vitro* by Song et al. [43]. Notably that some researchers evaluated the antiradical activity of the *Thymus* extracts with regard to their high polyphenolic contents with the RA as a major component [30].

Conclusion

This conducted studies provides data on the chromatographic characterization of polyphenols in the *S. hortensis* herb under cultivation conditions of western Ukraine. The specific 'chromatographic fingerprints' and presence of the RA, CA and ChA were detected by the HPTLC method. The contents of five hydroxycinnamic acids (RA, ChA, CA, ferulic, and neochlorogenic) and nine flavonoids (catechin, quercetin, apigenin, luteolin, rutin, hyperoside, acacetin-7-*O*-glucoside, apigenin-7-*O*-glucoside, and luteolin-7-*O*-glucoside) were evaluated using HPLC. Among them the significant amount of RA (21.42 mg/g) followed by the flavone derivatives and CA was found. Used chromatographic techniques could be regarded as the express-methods for the phytochemical identification of *S. hortensis* herb. As the investigated plant raw material contains a wide variety of bioactive polyphenols it can be useful for the development of new drugs and functional food additives.

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Table 1. Amounts of phenolic compounds in the *S. hortensis* herb evaluated by HPLC method

Compound	Retention time, min	Content, mg/g
Neochlorogenic acid	14.8	0.33±0.01
Catechin	19.5	0.21±0.01
ChA	20.4	0.62±0.02
CA	21.6	2.14±0.05
Rutin	30.9	0.22±0.01
Hyperoside	31.6	0.92±0.03
Ferulic acid	32.3	1.13±0.04
Luteolin-7-*O*-glucoside	33.1	3.44±0.08
Apigenin-7-*O*-glucoside	36.8	5.63±0.15
RA	37.8	21.42±0.46
Acacetin-7-*O*-glucoside	45.8	0.80±0.02
Quercetin	46.6	0.59±0.01
Luteolin	47.0	1.08±0.03
Apigenin	52.4	1.94±0.05

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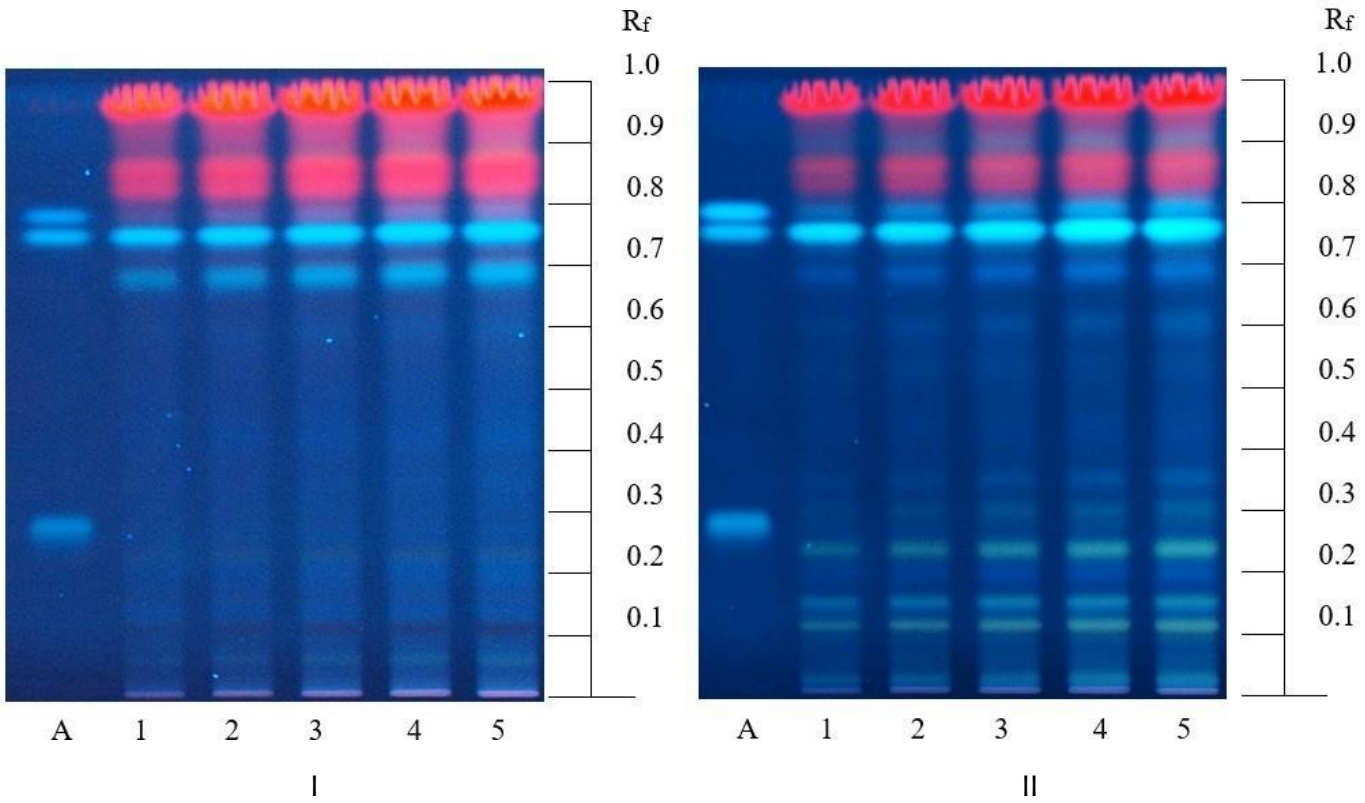

Figure 1. The typical HPTLC chromatograms of the methanol extract of *S. hortensis* herb (1–5) and standards of polyphenols (A –ChA, RA and CA with increasing R_f) before (I) and after (II) derivatization with 1% $AlCl_3$ at λ=366nm. Mobile phase: ethyl acetate: formic acid: water in a ratio 15:1:1

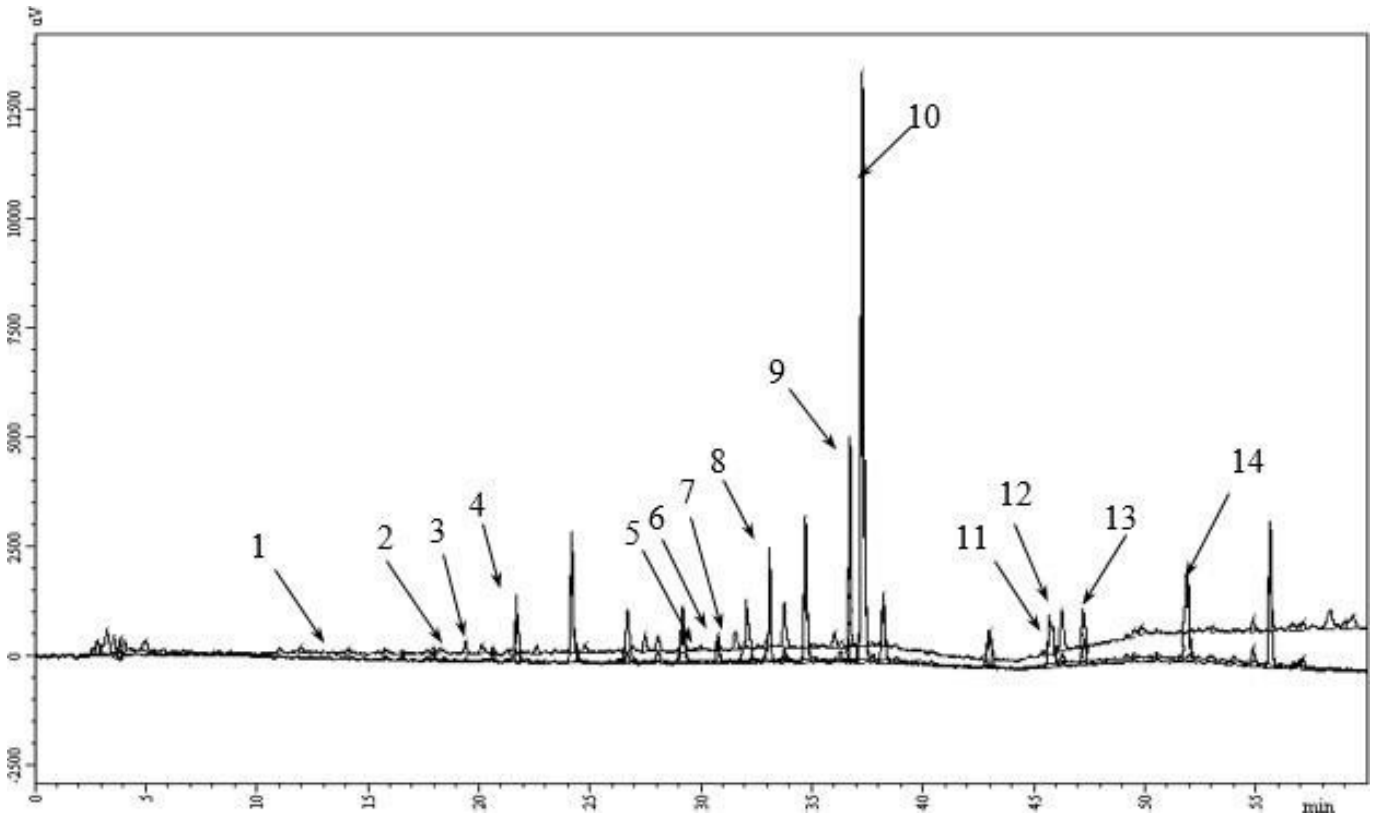

Figure 2. The typical HPLC chromatogram of phenolic compounds in the70% ethanol extract of *S. hortensis* herb:1 – neochlorogenic acid; 2 – catechin; 3 – ChA; 4 – CA; 5 – rutin; 6 – hyperoside; 7 – ferulic acid; 8 – luteolin-7-*O*- glucoside; 9 – apigenin-7-*O*-glucoside; 10 – RA; 11 – acacetin-7-*O*-glucoside; 12 – quercetin; 13 – luteolin; 14 – apigenin