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Effects of Eco-Agricultural Production of Phenolic Active Principles Synthesis in sect. Nepetoideae (Lamiaceae) Species

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[†]*This paper is dedicated to our beloved deceased Professor Biljana Božin, PhD*

Abstract. One way of cultivating different aromatic Lamiaceae species, especially those classified in the Nepetoideae section, is eco-agricultural production. This modern trend makes new products with less environmental pollution and a high value for human health and nutrition. Winter savory (Satureja montana), peppermint (Mentha piperita), thyme (Thymus vulgaris), and sage (Salvia officinalis) are highly important medicinal plants that were introduced and are being successfully cultivated in agricultural fields in Vojvodina. This study aimed to determine 10 phenolic compounds in ethanolic extracts of peppermint, sage, thyme, and winter savory cultivated according to eco-agricultural principles. The amount of examined phenolic compounds was measured using liquid chromatography method (HPLC-DAD). In all four extracts, rosmarinic acid (RA) was the most abundant compound, with the highest amount recorded in peppermint (12863.6 µg/g), followed by thyme (7083.76 µg/g). Other detected compounds ranged from 29.29 to 484.37 µg/g for chlorogenic acid, 22.67 to 979.77 µg/g for caffeic acid, 11.8 to 339.59 µg/g for ferulic acid, not detected (ND) to 45.93 µg/g for p-coumaric acid, 14.9 to 63.72 µg/g for cinnamic acid, ND to 68.13 µg/g for gallic acid, LOD to 125.03 µg/g for rutin, 38.98 to 82.75 µg/g for quercetin. Quercitrin was detected only in sage (50.78 µg/g). The results suggest that peppermint especially accumulates higher amounts of RA when cultivated under ecological conditions. Therefore, it represents a valuable resource of biologically active compounds, and the whole process could contribute to sustainable development if post-distillation waste material is used.
Keywords: Lamiaceae; ecological cultivation; phenolics; rosmarinic acid; HPLC.

Resumen. Una forma de cultivar diferentes especies aromáticas de Lamiaceae, especialmente aquellas clasificadas en la sección Nepetoideae, es la producción ecoagrícola. Esta nueva tendencia genera nuevos productos con menor contaminación ambiental y un alto valor para la salud y nutrición humana. La ajedrea (Satureja montana), la menta (Mentha piperita), el tomillo (Thymus vulgaris) y la salvia (Salvia officinalis) son plantas medicinales muy importantes que se introdujeron y se cultivan con éxito en los campos agrícolas de Vojvodina. Este estudio tuvo como objetivo determinar 10 compuestos fenólicos en extractos etanólicos de menta, salvia, tomillo y ajedrea cultivadas según principios ecoagrícolas. La cantidad de compuestos fenólicos examinados se midió utilizando el método de cromatografía líquida (HPLC-DAD). En los cuatro extractos, el ácido rosmarínico (AR) fue el compuesto más abundante, registrándose la mayor cantidad en la menta (12,863.6 µg/g), seguida del tomillo (7,083.76 µg/g). Otros compuestos detectados variaron de 29.29 a 484.37 µg/g para el ácido clorogénico, de 22.67 a 979.77 µg/g para el ácido cafeico, de 11.8 a 339.59 µg/g para el ácido ferúlico, de

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no detectado (ND) a 45.93 µg/g para el ácido p-cumárico, de 14.9 a 63.72 µg/g para el ácido cinámico, de ND a 68.13 µg/g para el ácido gálico, de LOD a 125.03 µg/g para la rutina, de 38.98 a 82.75 µg/g para la quercetina. La quercetina se detectó solo en salvia (50.78 µg/g). Los resultados sugieren que la menta, especialmente, acumula mayores cantidades de RA cuando se cultiva en condiciones ecológicas. Por lo tanto, representa una valiosa fuente de compuestos biológicamente activos y todo el proceso podría contribuir al desarrollo sostenible si se utilizan los residuos de la post-destilación.
Palabras clave: Lamiaceae; cultivo ecológico; compuestos fenólicos; ácido rosmarínico; HPLC.

Introduction

The Lamiaceae plant family, one of the most prominent plant families, is divided into two subfamilies: Nepetoideae and Lamioideae [1]. Plants from the Neptoideae subfamily are characterized by rosmarinic acid and a higher amount of essential oil [2]. On the other hand, plants from the Lamioideae subfamily contain iridoids and smaller amounts of essential oil [3]. However, plants from the Nepetoideae section possess the most significant pharmaceutical potential due to their expansive range of uses, including medicinal and aromatic properties [4]. They are also often used as culinary herbs, mainly for flavoring and food preservation. They are also used in condiments, beverages, aromatics, and cosmetics [5].

Species from this family show cosmopolitan distribution as they grow wild and are cultivated worldwide. Modern trends in agriculture, such as eco-agriculture, especially in medicinal and aromatic plant production, make new products less polluting and damaging to the environment and ecology, with a high value for human health and nutrition [6]. Eco-agriculture production preserves biological diversity and soil fertility, protects the environment, and, at the same time, improves the health and safety of produced herbs [7]. This production method involves pesticide-free cultivation but is not organic [8]. It was developed using organic fertilizer to support the farm's environmental sustainability [6]. It is characterized by diverse products with high quality, pests and weeds controlled without harmful ecological input, and improvement in soil quality [9]. These facts are of massive importance because medicinal plants are mainly consumed as tea or spices, row, without any chemical engineering processes. Consequently, herbal material produced in this way reaches a higher price on the processing market of medicinal plants and also in the production of preparations based on them (both drugs and dietary supplements).

In recent decades, the production of medicinal and aromatic plants has been an essential part of agriculture. However, nowadays, a relatively small part of arable land is covered with these plants when compared to areas used for producing cereals, fruits, vegetables, and industrial and fodder plants [10]. Due to the increased demand on the global market, the production of medicinal and aromatic plants also shows a growth tendency. Yet, despite favorable natural conditions, producers still fail to provide sufficient amounts of these plants. Floristic analysis of the Vojvodina region (Republic of Serbia) reveals the presence of around 650 plant species known for their medicinal potential. Although the demand, especially for some aromatic herbs, is constantly growing, numerous factors affect the quality of herbal material regarding pharmacologically active compound content. The concentration of phenolic compounds, which were the focus of this study, is influenced by climate conditions. Some plants, such as sage and winter savory, are more prone to reducing phenolic compounds' production. In contrast, peppermint and thyme produce considerable amounts of rosmarinic acid on Vojvodinian fields. However, only a few species are being cultivated regularly, and efforts are being made to promote and introduce important medicinal and aromatic herbs to the fields [11]. Winter savory (Satureja montana L.), peppermint (Mentha piperita L.), thyme (Thymus vulgaris L.), and sage (Salvia officinalis L.), though some native to the Mediterranean region are promising examples of highly important medicinal plants that were introduced and are being successfully cultivated on fields in Vojvodina. Food- and pharmaceutical products derived from these plants are available worldwide in the form of teas, tea mixtures, various types of dietary supplements, and herbal remedies and are widely used by the general population [12]. Active principles of these plants exhibit, among others, vigorous antibacterial, antifungal, and antioxidant activities [1]. Furthermore, these species are used to extract and isolate a significant number of biologically active compounds, such as rosmarinic acid, chlorogenic acid, and rutin [13]. Although numerous representatives of the Lamiaceae family, especially those belonging to the Nepetoideae subfamily, are characterized by the essential oil, the high content of various phenolic compounds, which contribute to overall medicinal potential, must not be neglected. Namely, rosmarinic acid, one of the most important phenolic

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compounds, is a promising agent for various ailments [14]. However, the percentage of produced rosmarinic acid and other active principles vary due to numerous environmental (photoperiod, cold stress, and soil type) as well as biotic factors (phenophase of collected plant material), which could be reduced using controlled eco-cultivation. Consequently, plant material of uniform quality would lead to better quality control of produced extracts in the pharmaceutical industry [15]. Therefore, the aim of this study was detailed chemical profiling of water-alcoholic liquid extracts of winter savory, mint, thyme, and sage cultivated according to eco-agricultural principles as well as evaluation of positioning of these extracts in comparison to literature data of extracts obtained from wild-growing plants or cultivated regularly.

Experimental

Plant material and preparation of extracts

Four medicinal crops from the Lamiaceae family (sect. Nepetoideae) – winter savory cv. Domaći (S. montana), peppermint cv. Danica (M. piperita), thyme cv. N-19 (T. vulgaris), and sage cv. Primorska (S. officinalis) – were cultivated at the Institute of Field and Vegetable Crops in Novi Sad, Alternative Crops Department, located in Bački Petrovac (45°21'N, 19°35'E). Voucher specimens were confirmed and deposited at the BUNS Herbarium under the numbers 2-1561, 2-1530, 2-1557, and 2-1548, respectively [16].

The selected plants were planted in spring 2017 using seedlings for winter savory, thyme, sage, and stolons for mint. The experiment was conducted on carbonate chernozem soil, and planting was done with a 70 cm row spacing for all plants to enable mechanical weed control. The application of nutrients is harmonized with soil fertility, and irrigation is performed as needed. Neither foliar fertilization nor plant protection agents were used. The experimental plot size was approximately 10m2 (5 rows, 3m long). The harvest was conducted in the second year of cultivation (2018), at the optimal development stage for each species, which occurs during July under the agroecological conditions of Serbia. The plant material was cut approximately 5 cm above the ground (one row per repetition, with the two marginal rows excluded from the test). The harvested plant material was then transferred to a solar dryer, where the temperature did not exceed 40 °C until constant weight was achieved (within 3 days). Dry plant materials were ground to a size of 200 mesh and placed into percolation devices. Extracts were obtained by triple percolation with 40 % ethanol at a drug/solvent ratio 1:4 for 72 hours.

Chemicals and reagents

Methanol and formic acid (HPLC grade) were purchased from Merck KGaA (Darmstadt, Germany). Standard substances including gallic acid (GA), caffeic acid (CA), chlorogenic acid (CHA), ferulic acid (FA), rutin (R), rosmarinic acid (RA), p-coumaric acid (pQA), trans-cinnamic acid (CNA), quercetin-dihydrate (Qe) and quercitrin (Qt) were purchased from Sigma-Aldrich GmbH (Sternheim, Germany).

Preparation of standard solutions

A stock standard solution containing a mixture of GA, CA, CHA, FA, RA, pQA, CAN, R, Qe, and Qt at a concentration of 1 mg/mL was prepared in methanol. A series of working solutions ranging from 0.18 – 36 µg/mL were prepared by dilution of stock standard solutions with methanol.

Sample preparation

Each liquid extract in 2.5 g was evaporated and redissolved in 5 mL of methanol. Before injection, the sample solutions were filtered through a 0.45 µm membrane PTFE filter (Rotilabo-Spritzenfilter 13 mm, Roth, Karlsruhe, Germany). The amounts of quantified phenolics and flavonoids were expressed as µg/g of liquid ethanolic extract and µg/g of dry extract (after quantification of dry extract yield).

Instrumentation and analytical conditions

HPLC was performed on Nucleosil C18 4.6 µm, 250 mm column by Agilent 1100 series instrument equipped with a diode array detector (DAD). The solvent gradient was performed by varying the proportion of solvent A (1 % formic acid in water (v/v)) to solvent B (methanol) as follows: initial 10 % B (flow 1 mL/min); 10 min, 25 % B (flow 0.8 mL/min); 20 min, 45 % B (flow 0.7 mL/min); 35 min, 70 % B (flow 0.7 mL/min); 40 min, 100 % B (flow 1mL/min). The total running time, including post-run time, was 48 min. The column temperature

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was maintained at 30 ºC, while the injection volume was 10 µL. The elution of compounds of interest was monitored at 280, 330, and 350 nm. The UV λmax for gallic, caffeic, and trans-cinnamic acids was 280 nm for p-coumaric, chlorogenic, rosmarinic, ferulic, and quercetin at 330 nm. In comparison, rutin and quercitrin were monitored at 350 nm.

Method validation

The developed method was validated in terms of linearity, repeatability, reproducibility, recovery, limit of detection (LOD), and limit of quantification (LOQ). Linearity was established by least-squares regression analysis of the results obtained after injection of six calibration standards mixture of increasing concentration in duplicate. The goodness-of-fit of the data was established by the determination of the linear regression coefficient (R2). Recoveries were assessed by spiking each extract at two concentration levels with a mixture of standards. The precision of the method was estimated through repeatability and reproducibility expressed as relative standard deviations (RSDs) of six replicates performed on the same day (intra-day) and three different days (inter-day) at three concentration levels for each compound. Limits of detection and quantification were determined as three and 10 standard deviations (SD) obtained after injection of natural samples spiked with low concentrations of analytes. These values were considered acceptable when the signal-to-noise ratio was ≥3 for LOD and ≥10 for LOQ.

Data processing

The results were processed using Microsoft Office Excel v2016 and Statsoft Statistica v12.5 software packages. The differences between analyzed extracts regarding the quantity of secondary metabolites were tested by the Kruskal-Wallis test, followed by the posthoc Dunn test. In contrast, the level of significance was kept at p= 0.05.

Results and discussion

Validation results

The applied chromatographic elution protocol separated peaks of interest well in the examined samples (Figures 1 and 2), enabling simultaneous quantification of ten phenolic compounds with suitable sensitivity, accuracy, and precision. Validation data are summarized in Table 1.

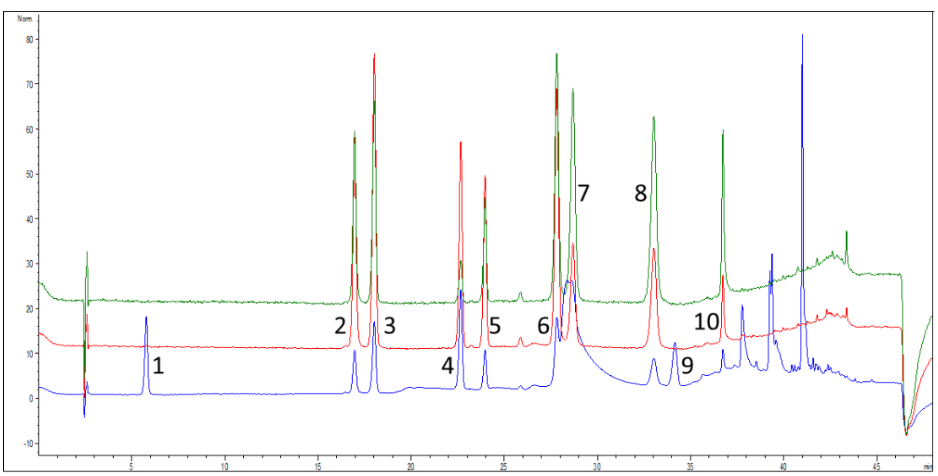

Fig. 1. HPLC-DAD chromatogram of calibration standards mixture (c = 8 µg/mL) with detection at 280 nm (blue), 330 nm (red) and 350 nm (green): 1 – gallic acid, 2 - p-coumaric acid, 3-quercetin, 4 -caffeic acid, 5 - chlorogenic acid, 6 - rosmarinic acid, 7 – rutin, 8 – quercetin, 9 - *trans*-cinnamic acid, 10 – ferulic acid.

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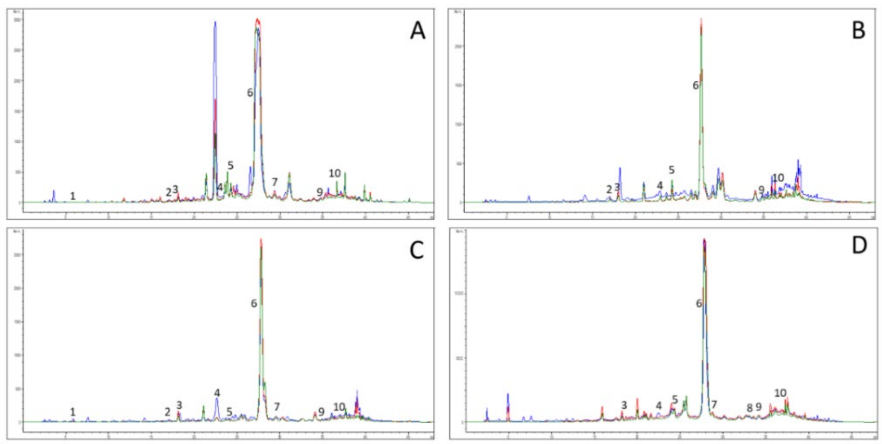

Fig. 2. HPLC-DAD chromatograms of analyzed liquid extracts of *M. piperita* **(A)**, *S. montana* **(B)**, *T. vulgaris* **(C)**, and *S. officinalis* **(D)** with detection at 280 nm (blue), 330 nm (red), and 350 nm (green). Detected compounds: 1 – gallic acid, 2 - p-coumaric acid, 3-quercetin, 4 -caffeic acid, 5 - chlorogenic acid, 6 - rosmarinic acid, 7 – rutin, 8 – quercetin, 9 - *trans*-cinnamic acid, 10 – ferulic acid.

Table 1. Analytical method validation data.

Analyte	Equation	R^2	Accuracy (recovery, %)	Precision RSD (%)		LOD (µg/g)	LOQ (µg/g)	Linearity range (µg/mL)	U* (%)
				Intra-day	Inter-day				
RA	y=3.5125x+0.3322	0.9983	91.20-101.47	1.33	1.27	0.15	0.50	0.5-30.0	6
CHA	y=2.9003x+4.6876	0.9995	100.71-101.32	4.50	1.40	0.15	0.30	0.3-30.0	5
CA	y=6.4807x+1.4903	0.9999	100.17-100.46	1.70	1.19	0.05	0.20	0.2-12.0	5
FA	y=1.6072x-5.5118	0.9979	96.96-101.38	0.95	0.64	0.10	0.20	0.2-36.0	6
pQA	y=5.5894x+12.473	0.9901	94.65-102.41	1.70	2.01	0.05	0.20	0.2-12.0	10
CNA	y=7.9398x+6.5653	0.9997	101.82-106.44	1.34	1.77	0.05	0.10	0.1-6.0	11
GA	y=3.0497+5.4495	0.9986	90.07-101.44	1.19	2.33	0.10	0.30	0.3-18.0	15
R	y=2.1225x+5.4787	0.9959	99.56-104.91	1.46	1.38	0.20	0.50	0.15-30.0	8
Qe	y=9.4489x+8.399	0.9981	97.61-102.78	1.42	1.24	0.05	0.20	0.18-10.71	7
Qt	y=2.4017x+2.8372	0.9996	98.39-99.87	1.80	0.94	0.15	0.50	0.5-30.0	5

*Expanded measuring uncertainty calculated with factor k=2

Analysis of samples

According to ethnobotanical studies performed throughout Serbia, Lamiaceae species and preparations based on them are the most frequent plant agents of traditional usage, including medicinal and culinary applications [17]. Their widespread use is supported by general recognition throughout history as safe and health-promoting. Still, nowadays, many scientific studies confirm the effectiveness and safety of these plants and their bioactive constituents [18]. The biological and medicinal potential of Lamicaeae species mostly correlates to the content of phenolic substances and

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essential oils, which are being extracted under various conditions, including different types of solvents and techniques such as maceration, ultrasonic extraction, extraction at elevated pressures, etc. [19-21] Beside essential oil characterization, the phenolic profile is of unquestionable interest for evaluating the pharmacological activity of medicinal plant raw materials. The chemical profiles of examined winter savory, mint, thyme, and sage ethanol extracts are presented in Table 2. In all four extracts, rosmarinic acid was the most abundant compound. The highest amount of rosmarinic acid was recorded in peppermint extract (12.86 mg/g), followed by thyme extract (7.1 mg/g). The lowest amount was characteristic of winter savory extract (4 mg/g). These results correspond to one of the previous chemical screening studies of 29 species belonging to the Lamiaceae family. However, the same investigation showed a higher amount of RA in Salvia officinalis extract when compared to *Mentha piperita* extract, which is not the case in this study [22]. The mint extract examined was also characterized by the highest amounts of phenolic acids (caffeic, chlorogenic, and ferulic acid) and rutin. However, the quantity quantified of rutin was significantly lower than previously reported [23].

The application of principal components analysis to a dataset containing results of detailed chemical characterization from the current study and results from previously conducted studies about investigated species shows that the first two Principal components (PCAs) describe around 60 % of samples' variability (Fig. 3). Results from previous studies, which were the basis for statistical analysis, are presented in Table 3 [21,23-41]. In terms of the first principal component (PCA1), the explained variability mostly correlates with the detected amounts of rosmarinic, gallic, and ferulic acids, while the amounts of chlorogenic acid and rutin dictate the shape of variability (PCA2). The position of the examined samples in the space defined by the first and second-factor axis indicates the separative grouping of processed samples. Namely, it can be concluded that most *S. officinalis* extracts usually contain lower amounts of chlorogenic acid and higher amounts of rutin, opposite to mint. However, the sage extract examined in this study (So1), obtained from ecologically grown *S. officinalis*, contained a lower amount of rutin. The explanation for this difference may be the impact of climate change on sage cultivation. In the studies above, plants were collected from their natural habitat, unlike plants in this research that were cultivated in Vojvodina, which is characterized by specific ecological abiotic factors. The same pattern can also be noticed in the case of *S. Montana* extract (Sm1), whose position is in the positive part of PCA2. On the other hand, thyme (Tv1) and peppermint (Mp1) extracts show separative grouping about other samples, mainly as a result of caffeic and rosmarinic acids amount, which allows us to hypothesize that the ecological cultivation of mint favorizes accumulation of RA. Rosmarinic acid, the most abundant in all extracts, has exciting properties. These properties have led to various applications, from food preservatives to cosmetics [42]. Different studies have shown that RA possesses more antioxidant activity than vitamin E, enabling its use as a stabilizer in natural cosmetics products [42,43]. Also, RA has been reported to have some important biological activities such as antiviral, antibacterial, and anticancerogenic [44,45]. In *in vivo* studies, it has been noticed that RA exhibits anti-allergic, anti-thrombotic, anti-inflammatory, and anticarcinogenic properties as well [46,47]. The inhibition of cyclooxygenase, especially inducible COX-2 isoform, whose expression and activity can be stimulated by various carcinogens, growth factors, inflammatory cytokines, and tumor promoters, is significant in terms of antiinflammatory action but also in cancer prevention [48]. Interestingly, RA and rosmarinic acid-rich extracts protect the skin from the oxidative stress induced by UVA radiation and thus may find their usage in photoprotective dermo-cosmetic preparations [49].

Because of the high content of RA, ethanolic extracts obtained from examined plants can present a good way to consume rosmarinic acid and achieve its benefits for the body. Consuming mint supplements containing phenolic compounds can lead to various benefits because of the highest content of RA and other phenolic acids, such as chlorogenic and ferulic acids. Chlorogenic acid (CHA) is an essential dietary polyphenol that possesses several important therapeutic activities such as antioxidant activity, antibacterial, hepatoprotective, cardioprotective, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, antiviral, anti-microbial, anti-hypertension, and a central nervous system stimulator. Furthermore, it has been found that CHA could modulate lipid and glucose metabolism. The hypocholesterolemic influence of CHA may result from the altered metabolism of nutrients, including amino acids, glucose, and fatty acids [50]. Ferulic acid (FA) is another phenolic acid with high medical potential. Based on preclinical research, FA has been suggested as a potential treatment for many health problems, including Alzheimer's disease, cancer, cardiovascular disorders, diabetes mellitus, and skin disease [51]. Also, flavonol rutin has demonstrated several pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective, and cardioprotective activities, and is also present in a notable concentration in the mint extract [43]. This increases the potential benefits of this extract. Furthermore, it must not be neglected that the examined aromatic plants are widely used to isolate essential oil, where valuable phenolic compounds remain unused. It has been suggested that the plant waste material remaining after the isolation of essential oil could be used to isolate various phenolic compounds, especially rosmarinic acid [28]. In conclusion, it can be stated that medicinal and aromatic plants, especially peppermint and thyme, are highly suitable for ecological cultivation in Vojvodina. This may represent an adequate response to worldwide

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increased demand for such species. Furthermore, the results suggest that mint mainly accumulates higher amounts of rosmarinic acid when cultivated under ecological conditions. Although these plant species are extensively studied and well-documented, it is recognized that their secondary metabolite content can vary significantly based on factors such as environmental conditions, geographical origin, and cultivation methods. This variation in chemical composition ultimately influences the medicinal potential of the plant. The innovation of this study lies in comparing the chemical profiles of plants grown using a novel cultivation approach—one that excludes pesticide use while promoting sustainability and environmental stewardship—against those from wild sources or alternative cultivation practices. Considering that evaluated species are mainly being used for essential oil extraction, in this case, the post-distillation waste material would represent a valuable resource of biologically active compounds, and the whole process would contribute to sustainable development.

Table 2. Chemical profiles of examined winter savory, mint, thyme and sage liquid ethanolic extracts (different small Latin letters indicate statistically significant differences at p=0.05 level).

Analyte	***Satureja montana* - Sm1**	***Mentha piperita* - Mp1**	***Thymus vulgaris* - Tv1**	***Salvia officinalis* - So1**
	(µg/g extract)			
RA	4011.4±240.68^{a}	12863.6±771.82^{b}	7083.76±425.02^{c}	5432.4±325.94^{d}
CHA	239.85±11.99^{a}	484.37±24.22^{b}	29.29±1.46^{c}	98.87±4.94^{d}
CA	31.17±1.56^{a}	979.77±48.99^{a}	334.07±16.70^{a}	22.67±1.13^{a}
FA	90.54±5.43^{a}	331.59±19.89^{b}	11.8±0.71^{c}	45.82±2.75^{d}
pQA	45.93±4.59^{a}	29.53±2.95^{b}	16.99±1.70^{c}	<LOD^{d}
CNA	63.72±7.01^{a}	23.13±2.54^{b}	62.74±6.90^{a}	14.9±1.64^{c}
GA	<LOD^{a}	0.42±0.07^{b}	68.13±10.22^{c}	<LOD^{a}
R	<LOD^{a}	125.03±10.00^{b}	40.35±3.23^{c}	114.76±9.18^{b,d}
Qe	70.64±4.94^{a}	71.85±5.03^{a}	82.75±5.79^{a}	38.98±2.74^{b}
Qt	<LOD^{a}	<LOD^{a}	<LOD^{a}	50.78±2.54^{b}
Dry extracts yield (%)	6.82	15.33	7.21	14.1

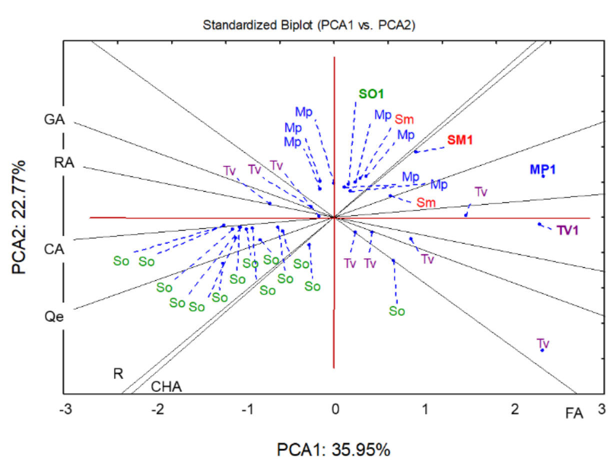

Fig. 3. Principal component analysis biplot.

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Table 3. Input data for principal component analysis.

μg/g d.e.	RA	CHA	CA	FA	pQA	pCA	tCNA	GA	R	Qe	Qt	Ref.	Origin of plant material
S. montana	1826.2	NDt	2079.6	726.9	NDt	NDt	25.8	772.05	5681.2	1200.5	NDt	[24]	Cultivated in an experimental farm
	17000	NDt	ND	NDt	100	NDt	100	NDt	500	1500	NDt	[25]	Wild growing plants
	NDt	NDt	65.95	25.2	24.1	NDt	NDt	NDt	NDt	NDt	NDt	[26]	Wild growing plants
	NDt	NDt	NDt	NDt	NDt	NDt	NDt	NDt	12160	NDt	NDt	[27]	Wild growing plants
	58818.18	3516.86	457.04	362.16	183.72	183.72	254.88	ND	ND	282.56	ND	TS	Eco-agricultural production
M. pipreita	10190	560	2530	180	NDt	NDt	NDt	130	1250	ND	NDt	[28]	Cultivated
	12400	730	1860	ND	NDt	ND	NDt	ND	2180	840	NDt	[23]	Cultivated
	35330	NDt	1460	NDt	NDt	NDt	NDt	NDt	NDt	NDt	NDt	[29]	Cultivated
	NDt	NDt	NDt	NDt	NDt	NDt	NDt	NDt	6080	NDt	NDt	[27]	Wild growing plants
	NDt	1074.5	483.5	215.5	NDt	NDt	NDt	ND	560	ND	NDt	[30]	Wild growing plants
	NDt	NDt	NDt	17.5	NDt	31	NDt	NDt	621.5	NDt	ND	[31]	Cultivated in an experimental farm
	19085	NDt	271	NDt	NDt	ND	NDt	ND	NDt	NDt	NDt	[32]	Comercially available
	83911.29	3159.62	6391.19	2163.01	192.63	192.63	150.9	2.74	815.59	468.69	ND	TS	Eco-agricultural production
T. vulgaris	94200	560	900	8640	180	NDt	NDt	1004.18	14160	830	NDt	[28]	Cultivated
	534.36	ND	102.2	267.18	70.08	NDt	2083.42	ND	NDt	NDt	NDt	[21]	Cultivated
	56620	NDt	1460	NDt	NDt	NDt	NDt	NDt	NDt	NDt	NDt	[29]	Cultivated
	NDt	NDt	NDt	NDt	NDt	NDt	NDt	NDt	14400	NDt	NDt	[27]	Wild growing plants
	14720	NDt	590	NDt	NDt	NDt	NDt	NDt	NDt	NDt	NDt	[33]	Cultivated

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	ND	NDt	58	ND	NDt	12.00	NDt	ND	NDt	NDt	NDt	[34]	Comercially available or wild growing plants
	21861	NDt	1215	NDt	NDt	400	NDt	375	NDt	NDt	NDt	[32]	Comercially available
	NDt	NDt	5170	905	NDt	ND	NDt	NDt	NDt	NF	NDt	[35]	Comercially available
	5700	ND	550	ND	NDt	180	NDt	NDt	NDt	NDt	NDt	[36]	Comercially available
	98249.10	406.24	4633.43	163.66	235.64	235.64	870.18	944.94	559.64	539.79	ND	TS	Eco-agricultural production
	1035.3	70.76	114.84	1089.82	69.6	NDt	149.06	ND	NDt	NDt	NDt	[21]	Cultivated
	34520	NDt	1460	NDt	NDt	NDt	NDt	NDt	NDt	NDt	NDt	[29]	Cultivated
	6500	NDt	520	NDt	NDt	NDt	NDt	NDt	NDt	NDt	NDt	[37]	Comercially available
	NDt	NDt	NDt	NDt	NDt	NDt	NDt	NDt	26880	NDt	NDt	[27]	Wild growing plants
	16330	NDt	680	NDt	NDt	NDt	NDt	NDt	NDt	NDt	NDt	[33]	Cultivated
	48656	NDt	1298.05	1220.5	NDt	NDt	NDt	18.7	NDt	NDt	NDt	[38]	Wild growing plants
	91890	ND	3475.2	1562.15	NDt	ND	NDt	94.1	NDt	NDt	NDt	[39]	Cultivated
S. officinalis	36434	783.5	594	NDt	NDt	562.5	NDt	NDt	NDt	NDt	NDt	[40]	Wild growing plants
	504.58	NDt	ND	ND	NDt	NDt	NDt	NDt	NDt	NDt	NDt	[41]	Cultivated in an experimental farm
	201.55	NDt	1.94	3.1	NDt	NDt	NDt	NDt	NDt	NDt	NDt	[41]	Cultivated in an experimental farm
	NDt	NDt	ND	49	NDt	ND	NDt	ND	NDt	NDt	NDt	[34]	Comercially available or wild growing plants
	6811	NDt	548	NDt	NDt	559	NDt	ND	NDt	NDt	NDt	[32]	Comercially available
	NDt	NDt	2960	135	NDt	103	NDt	NDt	NDt	1780	NDt	[35]	Comercially available
	5500	ND	510	50	NDt	ND	NDt	NDt	NDt	NDt	NDt	[36]	Comercially available
	38527.66	701.21	160.78	324.96	ND	ND	105.67	ND	813.9	283.5	360.14	TS	Eco-agricultural production

NDt − not determined; ND − not detected; TS – this study

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