

Analysis of polyphenols, phytosterols, and bitter principles in *Teucrium* L. species

Research Article

Renata Jurišić Grubešić^{1,*}, Dario Kremer², Sanda Vladimir-Knežević³,
Jadranka Vuković Rodrić¹

¹Department of Analytics and Control of Medicines,
University of Zagreb, Faculty of Pharmacy and Biochemistry,
HR-10000 Zagreb, Croatia

²Department of Pharmaceutical Botany with "Fran Kušan"
Pharmaceutical Botanical Garden, University of Zagreb,
Faculty of Pharmacy and Biochemistry,
HR-10000 Zagreb, Croatia

³Department of Pharmacognosy, University of Zagreb,
Faculty of Pharmacy and Biochemistry,
HR-10000 Zagreb, Croatia

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Abstract: Total polyphenols, tannins, β -sitosterol, and bitterness values were determined in native and cultivated populations of *Teucrium* L. species from Croatia (*T. arduini* L., *T. botrys* L., *T. chamaedrys* L., *T. flavum* L., *T. montanum* L., *T. polium* L., and *T. scordium* L. subsp. *scordioides* Schreb.). *Teucrium* species have long been present in folk medicine for diverse medicinal uses, but little is known about polyphenols, phytosterols, and bitter principles. Spectrophotometrically determined contents of total polyphenols (TP) and tannins (T) significantly varied among investigated *Teucrium* species and were somewhat higher in native populations. The highest TP and T contents were measured in native specimens of *T. montanum* (TP: 13.68%; T: 3.48%). Scanning densitometry was used for measurements of β -sitosterol levels in plant samples ranged from 0.056% (*T. montanum*) to 0.129% (*T. botrys*), and it was not significantly different between native and cultivated specimens of the same plant species. Bitterness values were similar for native and cultivated samples of the same plant species; the highest was measured for *T. montanum* (15659). The present study suggests that *Teucrium* species growing in Croatia have potential for cultivation and might be a valuable source of natural bioactive compounds.

Keywords: *Teucrium* • Total polyphenols • Tannins • β -sitosterol • Bitterness value • Multivariate analysis

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1. Introduction

Teucrium species have been used for centuries in folk medicine as cholagogic, diuretic, antispasmodic, and antidiabetic agents [1]; as antirheumatic, anti-inflammatory, antiseptic, anthelmintic, carminative and flavouring agents [2]; and as antipyretics and stimulants [3]. The genus *Teucrium* comprises approximately 200 herbs and shrubs spread all over the world, especially in the Mediterranean region, which has about 140 species. A few species are spread throughout South America, mountainous tropical Northeast and South Africa, and Australia [4]. According to Domac [5], 10 *Teucrium* species are growing in Croatia: *T. arduini* L., *T. botrys* L.,

T. chamaedrys L., *T. flavum* L., *T. fruticans* L., *T. marum* L., *T. montanum* L., *T. polium* L., *T. scordium* L. subsp. *scordioides* Schreb., and *T. scorodonia* L. Some of them are used in folk medicine in Croatia and Bosnia and Herzegovina: *T. arduini* for stomach diseases; *T. chamaedrys* for spleen diseases, cough, diarrhea, metabolic disorders, and as a diuretic; *T. marum* for gall diseases; *T. montanum* for liver and stomach diseases; *T. polium* for stomach diseases and *T. scordium* for diarrhea [6,7].

Previous chemical investigations of *Teucrium* species revealed the presence of flavonoids, steroidal compounds, volatile oil, tannins, and bitter principles (mostly diterpenoids) [8-17]. Quantitative analysis

* E-mail: rjurisic@pharma.hr

of micro- and macroelements [18,19], as well as determination of selenium [20] were also carried out in wild and cultivated specimens of *Teucrium* species growing in Croatia.

The purpose of this study was to perform multivariate comparison of polyphenol, phytosterol and bitter principle values in some *Teucrium* species growing in Croatia. Phenolic compounds have attracted a great deal of public and scientific interest because of their health-promoting effects as antioxidants. Plant sterols and stanols are known as cholesterol-lowering ingredients in foods and nutraceuticals, while bitter principles exhibit a significant gastro protective effect. This study may help define the chemotaxonomic relationships among different taxa of the complex genus *Teucrium*, which may help guide their medicinal use.

2. Experimental Procedures

The experimental part comprises extraction of plant material, spectrophotometric determination of total polyphenols and tannins, HPTLC-scanning densitometry for β -sitosterol analysis, as well as determination of bitterness value of examined plant decocts. Results obtained were evaluated using multivariate statistical analysis.

2.1 Apparatus

A Soxhlet apparatus was used for drug extraction. All absorbance measurements were carried out using an Agilent 8453 UV/Vis spectrophotometer (Agilent, Germany) equipped with PC-HP 845x UV-Visible System (Agilent, Germany) and 1 cm quartz cells.

Thin-layer chromatography (TLC) was carried out by applying solutions of the test substances to Kieselgel 60F₂₅₄ HPTLC plates (10×20 cm; Merck, Germany), using a Camag-Nanomat automatic spotter (Camag, Switzerland) equipped with calibrated, 1- μ l glass capillaries. The plates were scanned for absorbance at 495 nm using a Camag TLC scanner II (Camag, Switzerland).

2.2 Chemicals

Except for the Folin-Ciocalteu's phenol reagent (FCR), casein, and β -sitosterol (Merck, Germany), all the chemicals and reagents were of analytical grade and supplied by Kemika (Croatia). Double-distilled water was used throughout the work. Filtration of prepared sample solutions was performed using a 0.20- μ m Minisart-plus membrane filter (Sartorius AG, Germany).

2.3 Plant material

Native plants were collected during the blooming period at several locations in Croatia at the beginning of August

2006: *T. arduini* L. near Starigrad Paklenica (Velebit mountain) at an altitude of 600 m a.s.l., *T. botrys* L. in Zrmanja canyon (150 m a.s.l.), *T. chamaedrys* L. on the Gornje Jelenje pass at an altitude of 800 m a.s.l., *T. flavum* L. on the town Omišalj (Krk island) at s.l., *T. montanum* L. on the Gornje Jelenje pass at an altitude of 800 m a.s.l., *T. polium* L. near Garica (Krk island) at an altitude of 30 m a.s.l., and *T. scordium* L. subsp. *scordioides* Schreb. in Baška Draga (Krk island) at s.l. Among the species researched, *T. arduini* had the narrowest area of natural distribution, and it is endemic to Croatia, Bosnia and Herzegovina, Montenegro and northern Albania.

The cultivated samples were derived from the seeds of the same wild populations and cultivated under the same conditions in the Pharmaceutical Botanical Garden "Fran Kušan", Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia. Cultivated plants were also collected during the blooming period in July and at the beginning of August 2006. Voucher specimens (No. 9801–9812) are deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia.

Above-ground herbal parts of native and cultivated *Teucrium* species were dried for three weeks in a well-ventilated room, in one layer, protected from direct sunlight. To limit chemical oxidation and photo-oxidation, air-dried plant samples were placed in double paper bags labeled with the specimen number and sealed inside a dark container, which was stored in a dark, dry and cool place until analysis.

2.4 Analytical procedures

2.4.1 Total polyphenol and tannin analysis (FCR procedure)

Total polyphenol and tannin content in *Teucrium* species was determined spectrophotometrically using Schneider's method [21].

Stock solutions. Primary analyte stock solution was prepared by weighing exactly 10.0 mg tannin, dissolving in water and diluting to 100.0 ml with the same solvent. Secondary stock solution was made by mixing 5.0 ml of the standard solution and 5.0 ml of acetate buffer. FCR (0.5 ml) was added to different volumes of secondary stock solution of tannin (1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 ml, corresponding to 50, 40, 30, 20, 10, and 5 μ g of tannin, respectively). Each solution from step 1 was prepared in a 10-ml volumetric flask with 33% (w/v) Na₂CO₃·10H₂O. After filtration, the absorbance of the final blue solution was measured at 720 nm. Blank solution was prepared and measured identically, but without analyte.

Sample preparation. The mass of 0.250 g of powdered plant material (leaves, stems, and flowers) was extracted with 80 ml of 30% (v/v) methanol (70°C,

water bath, 15 min). After cooling and filtration, each extract was diluted to 100.0 ml with 30% methanol (basic sample solution, BSS). BSS (2.0 ml) was mixed with 8.0 ml of water and 10.0 ml of acetate buffer (solution 1, S1). S1 (10.0 ml) was shaken with 50.0 mg of casein during 45 min to allow adsorption of tannins, and then filtrated (solution 2, S2). S1 (1.0 ml) was mixed with 0.5 ml of FCR and diluted to 10.0 ml with 33% $\text{Na}_2\text{CO}_3 \cdot 10\text{H}_2\text{O}$. The same procedure was performed with S2. After filtration, the absorbance at 720 nm of the final blue solution was measured. Blank solution was prepared and measured identically, but without analyte.

The contents of total polyphenols and tannins were measured in three independent analyses. Absorbance values obtained for S1 correspond to total polyphenol content. The difference between the absorbance of S1 and S2 corresponds to the concentration of casein-adsorbed tannins in plant samples. The contents of total polyphenols and tannins were expressed as percentages of the dry mass of herbal material.

2.4.2 β -sitosterol analysis

The procedure was carried out according to Živanović *et al.* [22].

Sample preparation. The amount of 5 g of each powdered plant sample was weighed. Plant material was extracted with 20 g of petrol ether (Soxhlet apparatus, 6 h) and extracts were evaporated under vacuum; dry extracts were dissolved in 2 ml of a 1:1 mixture of chloroform-methanol mixture (test solutions) and analyzed by HPTLC and scanning densitometry. The reference solution was 10 mg/ml β -sitosterol dissolved in chloroform-methanol (1:1). HPTLC analysis was performed using Kieselgel 60F₂₅₄ HPTLC plates (10×20 cm). The mobile phase was a mixture of benzene and acetone (9:1, v/v). The samples were spotted on the HPTLC plates using the Camag-Nanomat automatic spotter in volumes of 5 μl (test solutions) and 1 μl (reference solution). Ascending chromatography was carried out in a twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for the mobile phase was 45 min at room temperature ($25 \pm 2^\circ\text{C}$). The length of chromatogram run was 175 mm. After development, the plate was allowed to dry at room temperature, after which it was sprayed with chlorosulfonic acid and heated for 5 min at 105°C in an oven. A Camag TLC scanner II was used for scanning densitometry analysis of the plate in absorbance mode at 495 nm (maximum absorbance). The content of β -sitosterol was expressed as a percentage of the dry mass of herbal material.

2.4.3 Bitterness value

The analysis was carried out according to the 9th edition of the German Pharmacopoeia (DAB 9) [23]. This analysis determines the bitterness value of a substance, which is defined as the reciprocal of the greatest dilution factor of a compound, liquid or extract that tasters still judge to be bitter. It is defined with respect to quinine hydrochloride, for which the bitterness value is set to be 200 000. A panel of six tasters was used, as recommended in the method. Panel members rinsed their mouths with water before tasting each sample. To correct for individual differences in tasting bitterness amongst the panel members, a correction factor was determined for each panel member (see below).

Quinine stock solution (QSS): This was prepared by dissolving 0.100 g of quinine hydrochloride in water and diluting to 100.0 ml. QSS (1.0 ml) was diluted to 100.0 ml with water.

Quinine reference solution (QRS): A series of dilutions was prepared by placing in a first tube 3.6 ml QSS and increasing the volume by 0.2 ml in each subsequent tube to a total of 5.8 ml; the content of each tube was diluted to 10.0 ml with water. The least concentrated solution that still had a bitter taste was determined as follows: each taster took 10.0 ml of the most dilute solution into the mouth and passed it from side to side over the back of the tongue for 30 s; if the taster did not consider the solution bitter, he or she spit it out, waited for 1 min, and then rinsed his or her mouth with water. After 10 min, the taster tried the next higher concentration.

The correction factor k for each panel member was calculated from the expression:

$$k = 5.00/n$$

n = number of milliliters of the QSS in the dilution of the lowest concentration that is judged to be bitter. The average value of correction factor k in the experiment was 1.04.

Tasters who were unable to taste any bitterness when using the reference solution prepared from 5.8 ml of stock solution were excluded from the panel.

Sample preparation. Boiling water (1000 ml) was added to 1.0 g powdered plant material, and heated for 30 min in a water bath with continuous stirring. After cooling, decoct was filtered and diluted to 1000 ml with water.

The extracts of plant samples containing bitter principles were prepared as the series of dilutions by placing in a first tube 0.5 ml of extract and increasing the volume by 0.1 ml in each subsequent tube to a total volume of 2.0 ml; the content of each tube was diluted to 10.0 ml with water. Bitterness of the prepared extracts was examined in the same way as bitterness of the QSS. Bitterness values were expressed as the reciprocal of the lowest concentration (g/ml) of the extract that tasters

considered to have bitter taste, divided by correction factor k .

2.5 Statistical analysis

The contents of total polyphenols, tannins, and phytosterols, as well as the bitterness values of native and cultivated populations of *Teucrium* L. species were analyzed using a multivariate approach [24]. Cluster analysis was performed with the unweighted pair-group method with arithmetic mean (UPGMA) using Euclidean distance (D_E). UPGMA generally yields the most accurate results for classification purposes [25]. To confirm the results of UPGMA, principal component analysis (PCA) was used. PCA calculations were based on the correlation matrix between the values of the characteristics, which means that the contribution of each variable was independent of the range of its values [26–28]. Statistical analysis of the experimental phytochemical results was carried out using Statistica 7 (StatSoft Inc., Tulsa, OK, USA).

3. Results and Discussion

Spectrophotometric determination of total polyphenols and tannins was performed using Folin-Ciocalteu's phenol reagent (FCR) [21] and comprehensive validation of this method was carried out by authors Jurišić Grubešić *et al.* [29]. Table 1 shows the results of the determinations of total polyphenols (TP), tannins (T), β -sitosterol, and

bitterness values in above-ground herbal parts of native and cultivated populations of the following *Teucrium* L. species: *T. arduini* L., *T. botrys* L., *T. chamaedrys* L., *T. flavum* L., *T. montanum* L., *T. polium* L., and *T. scordium* L. subsp. *scordioides* Schreb.

Amounts of TP and T varied among the *Teucrium* species and were somewhat higher in native than in cultivated samples. The highest amounts of TP were found in native specimens of *T. montanum* (13.68%) and *T. polium* (10.80%). Cultivated samples of *T. arduini* contained the lowest TP percentage (6.40%). TP content in other native populations ranged from 8.20% to 8.80%, while it ranged from 7.60% to 7.90% in cultivated ones. The results obtained for tannins showed a similar pattern to those for TP. Native specimens of *T. montanum* and *T. polium* contained the highest level of tannins (3.48% and 2.16%, respectively), while in cultivated specimens T content ranged from 1.00% (*T. botrys*) to 3.12% (*T. montanum*). Generally, the largest differences in the contents of polyphenolic compounds were observed between native and cultivated specimens of *T. polium*. According to Maleš *et al.* [30], similar results were achieved for the content of polyphenolic compounds in *T. polium* from Lastovo island, Croatia (TP: 10.71% and T: 2.16%). It is well known that phenolic compounds have attracted a great deal of public and scientific interest because of their health-promoting effects as antioxidants. Our comparative phytochemical study confirms that some *Teucrium* species in Croatia are a rich source of polyphenols and valuable candidates for further investigation.

Species	Origin	Total polyphenols (%)	Tannins (%)	β -sitosterol (%)	Bitterness value
		$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
<i>T. arduini</i>	N	6.80 $\pm$ 0.03	1.40 $\pm$ 0.03	0.072 $\pm$ 0.000	7799 $\pm$ 166
	C	6.40 $\pm$ 0.02	1.32 $\pm$ 0.03	0.072 $\pm$ 0.001	7799 $\pm$ 166
<i>T. botrys</i>	N	8.20 $\pm$ 0.04	1.20 $\pm$ 0.02	0.129 $\pm$ 0.001	8013 $\pm$ 287
	C	7.60 $\pm$ 0.05	1.00 $\pm$ 0.01	0.129 $\pm$ 0.002	7799 $\pm$ 166
<i>T. chamaedrys</i>	N	8.20 $\pm$ 0.02	1.20 $\pm$ 0.05	0.078 $\pm$ 0.000	12912 $\pm$ 638
	C	7.80 $\pm$ 0.02	1.08 $\pm$ 0.03	0.079 $\pm$ 0.001	10228 $\pm$ 353
<i>T. flavum</i>	N	8.80 $\pm$ 0.06	1.68 $\pm$ 0.01	0.080 $\pm$ 0.003	5196 $\pm$ 105
	C	7.80 $\pm$ 0.05	1.40 $\pm$ 0.02	0.089 $\pm$ 0.001	5196 $\pm$ 105
<i>T. montanum</i>	N	13.68 $\pm$ 0.07	3.48 $\pm$ 0.03	0.056 $\pm$ 0.001	15659 $\pm$ 1102
	C	12.80 $\pm$ 0.05	3.12 $\pm$ 0.02	0.056 $\pm$ 0.000	14866 $\pm$ 899
<i>T. polium</i>	N	10.80 $\pm$ 0.04	2.16 $\pm$ 0.04	0.074 $\pm$ 0.002	12912 $\pm$ 638
	C	7.60 $\pm$ 0.03	1.60 $\pm$ 0.05	0.074 $\pm$ 0.002	12912 $\pm$ 638
<i>T. scordium</i> subsp. <i>scordioides</i>	N	8.40 $\pm$ 0.02	1.72 $\pm$ 0.02	0.080 $\pm$ 0.002	7799 $\pm$ 166
	C	7.90 $\pm$ 0.03	1.46 $\pm$ 0.03	0.078 $\pm$ 0.001	7799 $\pm$ 166

Table 1. Contents of total polyphenols, tannins, β -sitosterol, and bitterness values in native (N) and cultivated (C) populations of *Teucrium* species. Results show the mean and standard deviation (SD), $n=3$.

The validation of a HPTLC-scanning densitometry for determination of β -sitosterol was performed according to recommended methods of validation [31,32] and included evaluation of precision, accuracy, linearity, and limits of detection and quantification, except specificity. The linearity was tested by the method of the least squares using three replicates of ten different concentrations within the working range between 2.0–20.0 $\mu\text{g/ml}$ of β -sitosterol. The linear relationship obtained between the peak area (A) and the concentration of β -sitosterol (c) is described with the following regression equation $A=617.07c-55.61$ ($r^2=0.9979$), where the concentration of the standard is expressed in $\mu\text{g/ml}$. Instrumental precision expressed as relative standard deviation (RSD) was checked by repeated scanning ($n=6$) of the same spot of β -sitosterol (10 $\mu\text{g/ml}$) and RSD values were below 0.84%. Repeatability expressed as relative standard deviation (RSD) was checked by analyzing β -sitosterol (10 $\mu\text{g/ml}$) individually ($n=5$) and RSD values were below 1.26%. Inter-day precision was studied by analyzing 5 and 10 $\mu\text{g/ml}$ of β -sitosterol in triplicates on different days and RSD values were 1.06% and 2.35%, respectively. The detection and quantification limits were calculated on an S/N=3, for limit of detection, and S/N=10, for limit of quantification, basis. The limit of detection (LOD) value for β -sitosterol was found to be 0.3 $\mu\text{g/ml}$

and limit of quantification (LOQ) value was 1.0 $\mu\text{g/ml}$. The accuracy of the method was evaluated by spiking the sample with 10 $\mu\text{g/ml}$ of β -sitosterol. The recoveries were between 97.1 and 102.4%. The results of validation confirmed that proposed HPTLC-scanning densitometry method was reproducible. However, the specificity of the procedure for β -sitosterol is questionable, because of the possible interference of the other components of the plant (e.g. other sterols) on the result.

The results of β -sitosterol determination using HPTLC-scanning densitometry showed that the amounts of β -sitosterol were not significantly different between native and cultivated samples of the same plant species. The HPTLC-scanning densitometry example of β -sitosterol determination in *Teucrium* species is presented in Figure 1. *T. montanum*, for which the highest levels of TP and T were observed, contained the smallest amount of β -sitosterol (0.056%), while *T. botrys* had the highest content (0.129%). Other plant specimens were found to contain 0.072–0.089% β -sitosterol. To the best of our knowledge, only one study covering determination of phytosterols in *Teucrium* species was published. The seed oil of three *Teucrium* species (*T. alopecurus*, *T. nabli*, and *T. polium*) were analyzed by Hachicha *et al.* [33] and it was characterized by a high amount of phytosterol

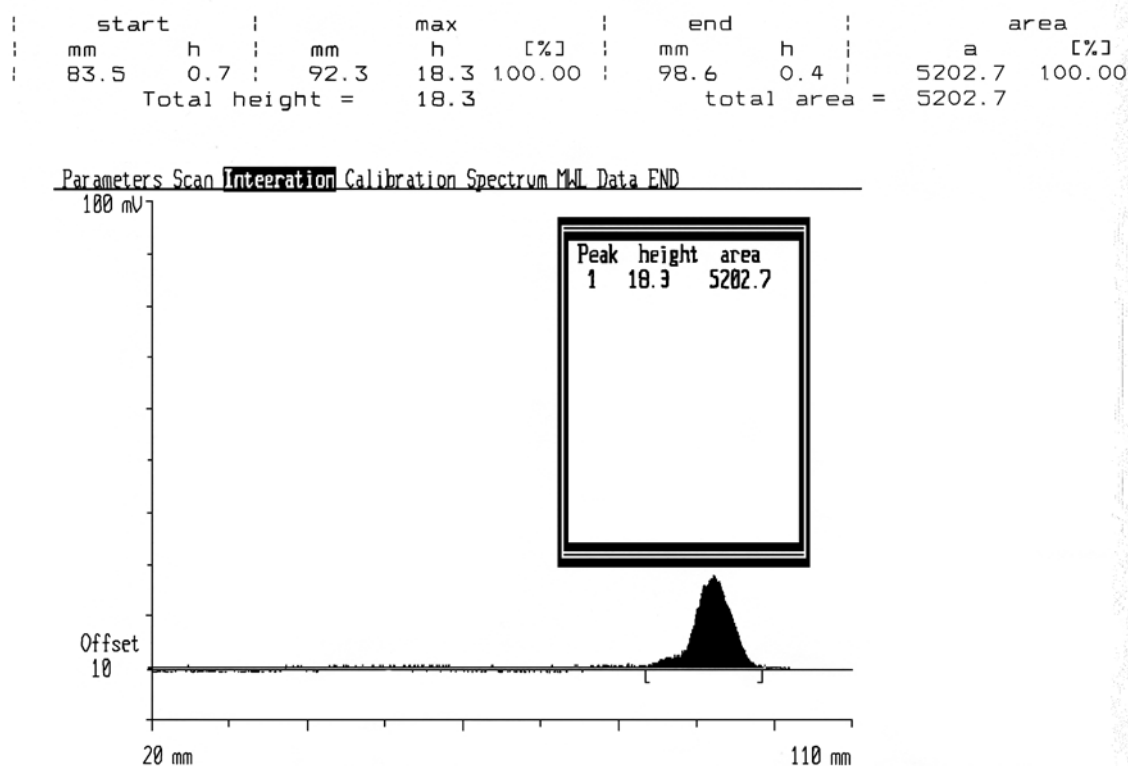


Figure 1. Densitometric scan of track of β -sitosterol standard solution.

fraction, wherein clerosterol, sitosterol, and stigmasterol were the main constituents. Phytosterols have been used as blood cholesterol-lowering agents for more than 50 years, since they inhibit cholesterol absorption in the small intestine. Therefore, phytosterols are recognized today as an important component of diets designed to reduce the risk of coronary heart disease [34,35]. Our results emphasize the importance of broad phytosterol investigations of *Teucrium* species in Croatia.

Bitterness value analysis determines the bitterness value of a substance, which is defined as the reciprocal of the greatest dilution factor of a compound, liquid or extract that tasters still judge to be bitter. Bitterness values of investigated plant extracts were generally similar for native and cultivated samples of the same *Teucrium* species. The smallest bitterness value was found for *T. flavum* (5196), while the highest one was found for *T. montanum* (15659 for native, 14866 for cultivated), followed by *T. polium* and *T. chamaedrys* (>10000). These findings are consistent with the common use of these *Teucrium* species for treating liver and stomach diseases.

The unweighted pair-group method with arithmetic mean (UPGMA) using Euclidean distance (D_E) was used to classify native populations of *Teucrium* species according to contents of total polyphenols, tannins, phytosterols and bitter principles (Figure 2A). The most similar native populations were *T. flavum* and *T. scordium* subsp. *scordioides*, with a Euclidean distance (D_E) of 0.71. *T. arduini* was connected to these two species at $D_E=1.05$. These three species were connected with a cluster formed by *T. chamaedrys* and *T. polium* ($D_E=1.97$). The most different species was *T. montanum*, connected to other species at $D_E=4.05$.

The same analysis of cultivated plants (Figure 2B) showed that the most similar were *T. arduini* and *T. scordium* subsp. *scordioides* ($D_E=0.80$). Only these two species formed a separate cluster. As was the case for native specimens of *T. montanum*, cultivated samples were the most different from other species ($D_E=4.39$).

Nearly all natural and cultivated plants of the same species were connected into one cluster (Figure 2C). The exception was *T. polium*, the cultivated population of which was connected with the native population of *T. chamaedrys* at $D_E=0.63$, while the native population of *T. polium* was connected to both populations of *T. montanum* ($D_E=2.19$). Natural and cultivated plants of *T. montanum* and natural populations of *T. polium* were the most different from all other species ($D_E=3.84$). These results are in accordance with the study of Pavlova and Vasileva [36], that also showed a great variability in morphological traits between different populations of *T. polium* from Bulgaria.

Principal component analysis (PCA) generally confirmed the results of UPGMA (Figure 3). The first principal component explains 74.54% of the total variance; the second, 15.08%; and the third, 9.60%. Thus, the first three components account for 99.21% of the variance, which points to the effectiveness of PCA in this type of study. Table 2 shows the eigenvector matrix with the loading of each variable in each principal component. Content of tannins and total polyphenols contributed the most to the first PC axis, while content of β -sitosterol contributed the most to the second PC axis. For PC 3, bitterness values gave the greatest contribution.

The results show that most of the species studied here had similar levels of the compounds analyzed, regardless of whether they were cultivated or natural. Therefore, it may be possible to cultivate the investigated species for commercial purposes without harvesting them in nature, which may prevent or reduce the danger of species extinction.

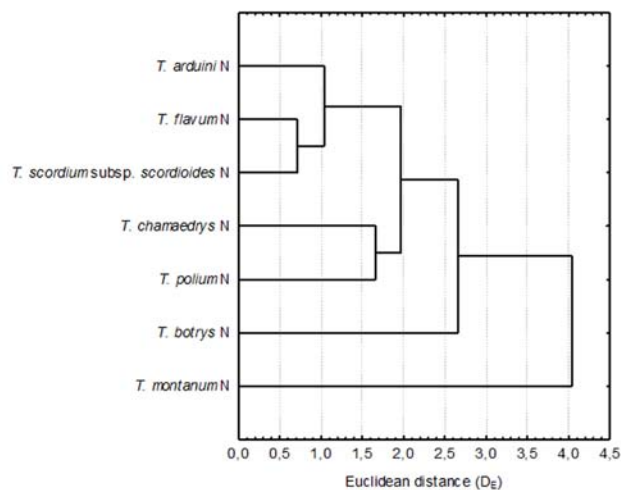
4. Conclusions

In conclusion, spectrophotometrically determined content of polyphenolic substances in investigated *Teucrium* species was somewhat higher in native samples than in cultivated ones. The contents of β -sitosterol and bitterness values were not significantly different for native and cultivated specimens of the same plant species. Multivariate analysis (UPGMA and PCA) showed that most of the natural and cultivated samples of the same species were clustered together. *T. montanum* appeared to be the most different from all other species and generally had the highest concentrations of bioactive compounds examined, which may explain its wide traditional therapeutic use in Croatia and neighboring regions. Although cultivation under conditions simulating the natural environment appeared to influence the levels of the bioactive compounds, the levels were nevertheless comparable between cultivated and natural *Teucrium* populations. The present study suggests that *Teucrium* species growing in Croatia have potential for cultivation and might be a valuable source of natural bioactive compounds.

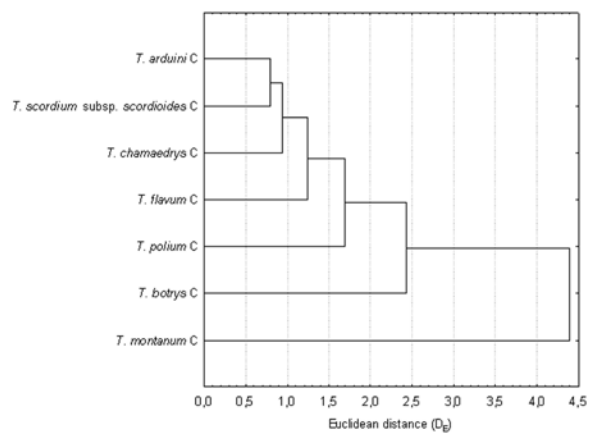
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A



B



C

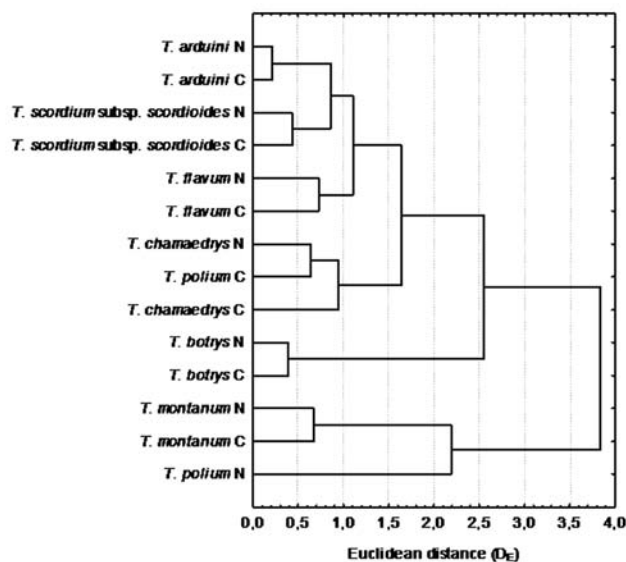


Figure 2. UPGMA dendrogram of native *Teucrium* species (A). UPGMA dendrogram of cultivated *Teucrium* species (B). UPGMA dendrogram of native and cultivated *Teucrium* species (C).

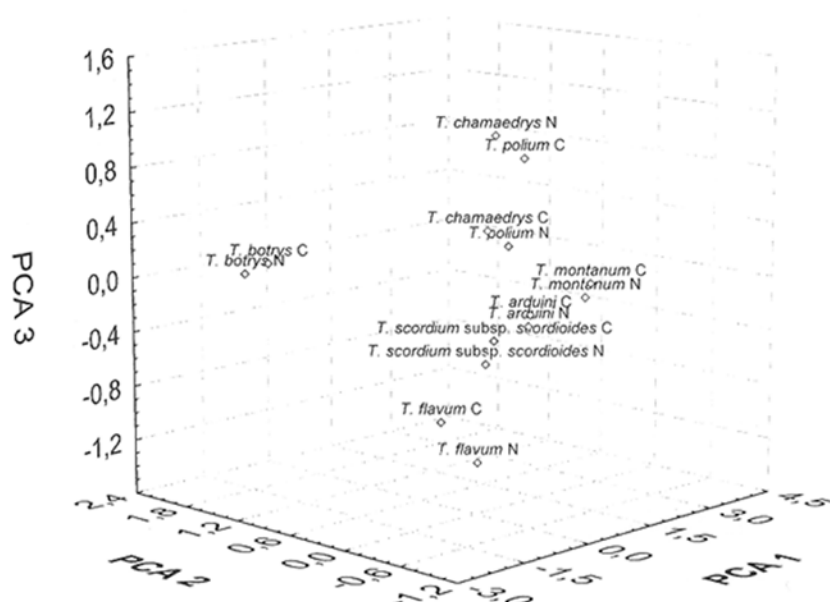


Figure 3. PCA of analyzed components in native and cultivated *Teucrium* species.

Variable	PC 1	PC 2	PC 3	PC 4
Total polyphenols	0.531332	0.424092	-0.305268	0.666816
Tannins	0.551523	0.119893	-0.422547	-0.709156
β -sitosterol	-0.420941	0.882435	0.045201	-0.205118
Bitterness value	0.486125	0.164559	0.852187	-0.101882

Table 2. Eigenvectors of the principal components (PCs) for native and cultivated *Teucrium* species.

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