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Chemical Profile, Elemental Composition, and Antimicrobial Activity of Plants of the *Teucrium* (Lamiaceae) Genus Growing in Moldova

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Abstract: *Teucrium* L. is a widely distributed genus often used for the treatment of digestive disorders and respiratory problems. The aim of the present study was to determine the chemical composition of essential oils and elemental content of the plant species *Teucrium polium*, *Teucrium hircanicum*, *Teucrium botrys*, *Teucrium chamaedrys*, *Teucrium flavum*, *Teucrium orientale*, and *Teucrium scordium* of Moldovan origin, as well as to evaluate the antimicrobial activity of their extracts. The composition of essential oils was determined using gas chromatography–mass spectrometry (GC–MS), and neutron activation analysis (NAA) was used to assess the elemental composition of plants. Antimicrobial tests were performed in vitro on the *Bacillus subtilis*, *Pseudomonas fluorescens*, *Xanthomonas campestris*, *Erwinia amylovora*, *Erwinia carotovora*, and *Candida utilis* strains using the double-dilution method. GC–MS allowed the identification of 59 components of the analyzed essential oils, and showed that the analyzed species belong to four different chemotypes. Using NAA, 18 major and minor elements, the contents of which fell within the value ranges reported for other medicinal herbs of this genus, were identified. The hydroalcoholic extracts from *Teucrium* spp. exhibited in vitro antibacterial and antifungal activity at 0.03–0.06% and 0.015–0.03%, respectively. The extracts from *Teucrium* spp. exhibited high antibacterial and antifungal activity, enabling their application for medical purposes.

Keywords: *Teucrium* L.; chemical composition; mineral composition; activation analysis; antimicrobial activity; essential oil; medicinal plants

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1. Introduction

Teucrium L. (Lamiaceae family) is a widely distributed genus including more than 300 species, of which one-sixth are found in the Mediterranean region [1,2]. The members of this genus mostly represent perennial herbs, shrubs, or subshrubs; they have erect or ascending leafy stems. Leaves are petiolate or sessile, not divided, with subentire-to-crenate–dentate margins, along with inflorescence-pedunculated cymes or verticillasters—distant, or condensed into spikes or heads—and ovoid or obovoid rounded nutlets. In the flora of the Republic of Moldova, the *Teucrium* L. genus is represented by six species [3].

Many species of the genus *Teucrium* L. are used in folk medicine—especially in the treatment of digestive disorders and respiratory problems. Externally, some Teucrium species can be useful in the treatment of purulent eruptions, furuncles, wounds, mycosis, and skin abscesses [4,5].

A large number of compounds—such as sesqui-, di-, and triterpenoids, iridoids, flavonoids, steroids, carbohydrates, polyphenols, phytosterols, phenolic acids, amino acids, tannins, vitamin C, saponins, and iridoid glycosides—with a wide spectrum of biological properties, have been isolated from *Teucrium* L. plants [6–8]. These constituents have been found to have hypoglycemic [9], hypolipidemic [10], antispasmodic [11], anti-inflammatory [12], analgesic [13], antipyretic, antifungal, antibacterial, antiviral [14], cytotoxic, detoxifying, hemostatic, cicatrizing, diuretic, and antiseptic activities [15,16]. Modern pharmacological studies suggest that some *Teucrium* L. species as are rich natural sources of anticancer compounds, which have proven to be effective against HCT-116 cells [17].

The analysis of essential oils of several *Teucrium* L. species, obtained via hydrodistillation or microwave-assisted hydrodistillation, has been performed by capillary gas chromatography (GC), gas chromatography–mass spectrometry (GC–MS), and gas chromatography–flame ionization detection (GC–FID) [18–20]. These oils include caryophyllene, caryophyllene oxide, α-humulene, germacrene D, α-pinene, β-pinene, α-muurolene, (E)-β-farnesene, and carvacrol [21,22].

The microbiological investigation showed that the essential oil of *Teucrium* L. species possesses antimicrobial activity against *Escherichia coli*, *Bacillus cereus, Staphylococcus aureus*, and the yeast *Saccharomyces cerevisiae* [23]; antioxidant activity based on in vitro assays such as free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric-reducing activity power (FRAP), and total antioxidant capacity (TAC) [24,25]; antiphytoviral activity against cucumber mosaic virus (CMV) [21], and antibacterial activity against 15 clinical isolates of *Klebsiella pneumoniae* [14].

Many *Teucrium* L. plants, due to their effective therapeutic options against microorganisms, are used as natural conserving agents in the food industry [26]. *T. polium* also has a pronounced efficacy against different species of insects [6]. In view of the aforementioned, *Teucrium* L. species have a significant medicinal and economic importance, and have been widely studied in recent decades.

This paper refers to seven medicinal native and allochthonous *Teucrium* species (*T. hircanicum*, *T. polium*, *T. botrys*, *T. chamaedrys*, *T. flavum*, *T. orientale*, and *T. scordium*) cultivated in the experimental fields of the National Botanical Garden (Institute), Chisinau, Republic of Moldova, on which information on chemical composition is lacking.

According to the purposes of this paper, the chemical composition of essential oils from the mentioned species of the *Teucrium* genus was evaluated by means of gas chromatographic techniques, and elemental content of the plants was determined using neutron activation analysis. Additionally, the antimicrobial assessment of hydroalcoholic extracts was performed on several fungi, as well as both non-pathogenic and phytopathogenic bacterial strains. It should be mentioned that this kind of analysis was performed for the first time for *Teucrium* plants of Moldovan origin.

2. Materials and Methods

2.1. Sample Collection and Extraction

The plant material—seven specimens of *Teucrium* spp. (aerial parts with inflorescences)—was collected in June 2018–2020 from the National Botanical Garden (Institute), Chisinau, Republic of Moldova, geographically located at latitude 46°58′25.43″ N and latitude 28°52′47.16″ E. The plants were cultivated in ecologically clean conditions. For analyses, only pure samples of plant material were used. Voucher specimens were deposited in the Herbarium of the Botanical Garden (Institute) (voucher copies are in SI). The identity of the species was established by Dr. Nina Ciocarlan (Department of Vegetal Resources).

Teucrium polium

***Teucrium polium* L.** (golden germander) is a perennial subshrub with tomentose, lanate or, rarely, pilose–hispid stems reaching ~30–50 cm in height. The opposite, simple, sessile, cuneate–oblong or linear leaves are ~0.7–2.5 cm in length. White-to-pale-cream-colored flowers form simple paniculate or corymbose inflorescences. The seeds are brown, reticulate nutlets.

Teucrium hircanicum

***Teucrium hircanicum* L.** (Caucasian germander) is a perennial species with hairy stems, and is woody at the base. Leaves are ovate or oblong–ovate, crenate, base truncate to cordate, or apex obtuse. The inflorescence is a dense terminal spike-like raceme around 15 cm long, and bracts are subulate. The calyx is campanulate, densely hairy, glandular–scaly, and prominently veined at fruiting. The corolla tube is hairy, limb-deflexed and concave, and reddish-purple. Nutlets are brownish, wider than long, and reticulate.

Teucrium botrys

***Teucrium botrys* L.** (cut-leaved germander) is an annual, many-branched plant with glandular–villosulous stems. Leaves are opposite, oval, simple, and deeply and ternately divided into short, obtuse, oblong segments. Flowers are usually 4–6 at each node, on pedicels of 4–8 mm, with the calyx strongly saccate at base—especially in age—eventually growing to 1 cm, with its lobes shorter than the tube, broadly triangular, and the lower two being somewhat smaller. The pink-to-purple flowers form whorls from the stem at the base of the leaves, and the fruit are nutlets.

Teucrium chamaedrys

***Teucrium chamaedrys* L.** (wild germander) is a perennial, evergreen plant with a creeping rootstock and upright-to-spreading stems that reach ~10–30 cm in height. The opposite oblong–ovate, deeply veined, pubescent, dark green leaves resemble those of a small oak tree. The tubular, labiate, rose-colored flowers are arranged in axillary whorls on leafy, terminal spikes. The seeds are ellipsoidal nutlets, with a dark reddish-brown and reticulate surface.

Teucrium flavum

***Teucrium flavum* L.** (yellow germander) is an evergreen, perennial, densely branched subshrub with flower stems up to 50 cm high, and is woody at the base. The leaves are opposite, ovate, dark green, and glossy above, with serrate margins. In inflorescences, the hermaphrodite flowers are arranged in whorls. The calyx is 8–9 mm long, hispid to villous hairy, and covered with glands. The corolla is cream to lemon-yellow, and sparsely hairy. The fruit are schizocarps consisting of four ovoid nutlets.

Teucrium orientale

***Teucrium orientale* L.** (Oriental germander) is a perennial species with a bushy appearance; its stems can be several or quite numerous, are 10–50 cm tall, and are slightly ascending at the base or erect. The leaves are spaced, broadly rhombic, ovate or almost round, thrice pinnately dissected into long and narrow linear lobes, somewhat laminar along the edge, green or greyish, and densely short haired. The lavender–blue flowers are small, tubular, one-lipped, and arranged in paniculate or paniculate–corymbose, usually elongated, often short-branched inflorescences. The nutlets are wrinkled, with small, transparent, granular glands.

Teucrium scordium

***Teucrium scordium* L.** (water germander) is a perennial stoloniferous plant. Stems are erect or ascending, sometimes purplish below, with no or few branches, and 10–30 cm height. Leaves are sessile, attenuate at base, oblong–elliptic, senate, or serrulate above, and apically rounded or acute; the leaves in the middle part of the stem are usually the largest. Flowers are whitish, pink, or purplish in the axils of the middle and upper leaves; verticillasters are 2–6-flowered; bracts are absent. Nutlets are subglobose, dark brown, and apically glandular.

2.2. Reagents and Apparatus

All of the reagents used in the present study were of analytical grade. To obtain essential oil samples, 250 g of aerial parts of the plant were hydrodistilled for 4 h in a Neo-Clevenger apparatus. * Distillates were supplementary extracted with diethyl ether and dried over anhydrous sodium sulfate, and subsequently applied for chromatographic measurements.

Average samples of dried plants were analyzed by neutron activation analysis (NAA).

The hydroalcoholic extracts were obtained using Soxhlet-type extractors for 4 h, then filtered and distilled under reduced pressure to dryness. In continuation, the crude extracts were used for the preparation of the antimicrobial assessment solutions. (The plant material of *T. scordium* was not sufficient for essential oil extraction and antibacterial tests).

2.3. GC–MS Analysis

The gas chromatographic analyses of the *Teucrium* sp. essential oils were performed in one repetition using an Agilent Technologies 7890A system with a 5975C Mass-Selective Detector (GC–MSD) equipped with a split–splitless injector (1 µL). The analysis was carried out on a fused silica capillary HP-5MS calibrated column (30 m × 0.25 mm i.d.; film thickness 0.25 µm). The injector and detector temperatures were kept at 250 °C. Helium was used as a carrier gas at a flow rate of 1.1 mL/min; the initial oven temperature program was 70 °C/2 min, which was then increased to 200 °C at the rate of 5 °C/min, and finally to 300 °C at the rate of 20 °C/min; the split ratio was 1:50. The MSD ionization energy was 70 eV, scan time was 1 s, acquisition mass range was from 30 to 450 amu, and solvent delay was 3 min [27].

2.4. Neutron Activation Analysis

The elemental composition of six *Teucrium* species was determined by neutron activation analysis at the IBR-2 reactor (Dubna, Russia). More details about sample irradiation and analysis can be found in [28,29].

The mass fractions of elements based on short-lived radionuclides—Mg, Al, Cl, Ca, and Mn—were determined by sample irradiation for 3 min at a neutron flux of 1.2×10^{12} cm^{-2} s^{-1} and measurement immediately after irradiation. To determine elements with long-lived isotopes—Na, K, Sc, Cr, Fe, Co, Zn, As, Br, Rb, Sr, Mo, Sb, Cs, and Ba—the cadmium-screened channel 1 was used. Samples were irradiated for 4 days at a neutron flux of 1.2×10^{11} cm^{-2} s^{-1} and measured twice after 4 and 20 days of irradiation for 20 min and 1.5 h, respectively. The analysis of the spectra was performed using the Genie2000 software, and the calculation of mass fractions was carried out using the software "Concentration" developed in FLNP.

The quality control of the measurements was ensured using the following certified reference materials of the National Institute of Standards and Technology: 1573—Tomato

leaves, 1575a—Pine needles, 1575a—Peach leaves, and 1633b—Coal fly ash. The experimentally obtained values were in good concordance with the certified ones.

2.5. Antimicrobial Activity Assessment

Antimicrobial activity assessments of the hydroalcoholic extracts from *Teucrium* species plants were performed on the non-pathogenic Gram-positive and Gram-negative strains of *Bacillus subtilis* NCNM-BB-01 (ATCC 33608) and *Pseudomonas fluorescens* NCNM-PFB-01 (ATCC 173 25323), respectively, phytopathogenic strains of *Xanthomonas campestris* NCNM BX-01 (ATCC 175 53196), *Erwinia amylovora* NCNM BE-01 (ATCC 176 29780), and *Erwinia carotovora* NCNM 177 BE-03 (ATCC 15713), and fungal strains of *Candida utilis* NCNM Y-22 (ATCC 44638).

For antimicrobial activity testing, the successive double-dilution method was used [30,31]. At the first stage 1 mL of peptone broth for test bacteria and Sabouraud broth for test candida were introduced into a series of 10 tubes; 1 mL of the analyzed mixture was introduced into the first test tube. Then, after agitation, 1 mL of the mixture was transferred to the next tube, and the procedure was performed until the 10th tube. In this way, the concentration of the initial solution was reduced two times in each subsequent tube. Simultaneously, the 24-h test microorganism cultures were prepared.

According to the McFarland index, the optical density (D.O.) of the suspensions for tested bacteria and fungi was 2.0 and 7.0, respectively.

Next, 1 mL of the microbial suspension was introduced to a tube with 9 mL of sterile distilled water. After mixing the contents of the tube, 1 mL of the obtained solution was transferred to the second tube of the five-tube series, each containing 9 mL of distilled water.

Then, 0.1 mL of the microbial suspension taken from the 5th tube was added to each tube with the titrated preparation, and the tubes with titrated preparation sand the seeded doses of the microorganisms were kept in the thermostat at 35 °C for 24 h. On the second day, preliminary analysis was performed. The last tube from the series, in which no visible growth of microorganisms was detected, was considered to be the minimal inhibitory concentration (MIC) of the preparation.

For the calculation of the minimum bactericidal and fungicidal concentrations, the contents of the test tubes with MIC and with higher concentrations were seeded on peptone and Sabouraud agar from Petri dishes. The seeded dishes were kept in the thermostat at 35 °C for 24 h. The concentrations of preparation that did not allow the growth of microorganisms were considered to be the minimum bactericidal and fungicidal concentrations of the preparation.

2.6. Statistical Analysis

In the present study, PCA was used in both Q and R modes to determine the similarities or dissimilarities not only between the investigated *Teucrium* species, but also between the mass fractions of all 18 elements quantified by INAA [30,32].

All data analyses were performed using StatSoftTH Statistica 11, OriginLab^{TM} OriginPro 2021, and PAST 4.03 [33,34] software.

3. Results and Discussion

3.1. GC–MS Identification of Essential Oil Constituents

Gas chromatography coupled with mass spectrometry analysis of essential oil samples from the *Teucrium* species revealed quantitative and qualitative differences between them. Thus, in *T. polium*, 34 components of the essential oil were detected, which corresponded to 94.42% of the total composition (Table 1). The main component (32.43%) of *T. polium* essential oil was germacrene D, followed by *β*-pinene (16.08%) and *β*-myrcene (7.63%), which is consistent with the literature data [21,23,33,35]. Lograda et al. [23] reported *a*-pinene (14.1–18%), *β*-pinene (15.1–18.1%), germacrene D (3.8–19%), and *β*-myrcene (8.2–10.4%) as major products of eastern Algerian populations of *T. polium*.

Table 1. Chemical composition of essential oil from *Teucrium* species grown in Moldova.

No.	RT * (min)	Component, Classification	Species					
			TP	TH	TB	TCH	TF	TO
1	4.28	α-Thujene (C_{10} **)	0.07	-	-	-	-	-
2	4.42	α-Pinene (C_{10})	5.27	-	3.03	1.69	12.38	-
3	4.72	Camphene (C_{10})	0.09	-	-	-	-	-
4	5.18	β-Thujene (C_{10})	0.16	-	-	-	-	-
5	5.27	β-Pinene (C_{10})	16.08	-	3.81	1.19	6.78	-
6	5.50	β-Myrcene (C_{10})	7.63	-	0.18	-	0.54	0.35
7	6.38	(+)-Limonene (C_{10})	5.82	-	3.29	-	4.64	-
8	6.55	(*E*)-β-Ocimene (C_{10})	0.23	-	-	-	2.87	2.35
9	6.81	(*Z*)-β-Ocimene (C_{10})	1.50	-	-	-	0.34	3.67
10	7.84	Terpinolene (C_{10})	0.11	-	-	-	-	-
11	8.11	β-Linalool (C_{10})	0.54	5.00	1.11	-	-	-
12	8.38	1-Octene-3-yl acetate	0.44	-	-	-	0.49	-
13	8.64	3-Octyl acetate	-	-	-	-	0.62	-
14	10.59	α-Terpineol (C_{10})	0.11	-	-	-	-	-
15	10.71	Myrtenal (C_{10})	0.14	-	-	-	-	-
16	12.03	2-Methylbutyl hexanoate	-	-	-	-	1.39	-
17	13.02	Bornyl acetate (C_{10})	-	-	0.61	-	-	-
18	14.65	α-Cubebene (C_{15})	-	-	-	-	0.42	3.02
19	15.35	Copaene (C_{15})	0.31	-	-	0.80	-	3.33
20	15.59	β-Bourbonene (C_{15})	0.37	-	0.38	1.68	1.60	0.39
21	15.74	β-Cubebone (C_{15})	0.17	-	-	1.81	-	9.61
22	15.75	β-Elemene (C_{15})	0.20	-	-	-	-	-
23	16.45	β-Caryophyllene (C_{15})	0.28	-	54.06	40.95	6.99	16.47
24	16.84	α-Bergamotene (C_{15})	-	2.18	-	-	0.36	0.77
25	17.02	β-Sesquiphellandrene (C_{15})	-	0.98	-	-	-	-
26	17.30	α-Caryophyllene (C_{15})	-	-	-	9.14	3.83	6.28
27	17.32	(*Z*)-β-Farnesene (C_{15})	6.90	30.44	0.70	-	-	-
28	17.40	Farnesane (C_{15})	-	2.49	-	-	-	-
29	17.48	Alloaromadendrene (C_{15})	0.30	-	15.81	0.78	0.39	0.35
30	18.00	α-Curcumene (C_{15})	-	0.81	-	-	-	-
31	18.02	Germacrene D (C_{15})	32.43	-	1.63	22.09	15.47	13.86
32	18.12	(+)-Valenune (C_{15})	0.28	-	-	-	-	-
33	18.28	(+)-Valencene (C_{15})	-	-	-	0.78	-	-
34	18.36	Bicyclogermacrene (C_{15})	7.44	-	1.60	1.65	0.79	3.95
35	18.57	δ-Guaiene (C_{15})	0.47	-	-	-	-	-
36	18.61	β-Bisabolene (C_{15})	-	0.88	-	-	29.96	-
37	18.69	Butylated hydroxytoluene	-	9.22	0.50	1.63	-	14.97
38	18.78	γ-Muurolene (C_{15}) α-Himachalene (C_{15})	0.67	38.21	-	-	-	-
39	18.87	Seline-3,7[11]-diene (C_{15})	-	-	-	5.98	-	0.57
40	18.97	(-)-β-Cadinene (C_{15})	0.34	-	0.58	6.55	0.70	3.79
41	19.18	α-Patchoulene (C_{15})	-	4.85	-	-	-	-
42	19.79	γ-Elemene (C_{15})	0.08	-	-	-	-	0.17
43	20.28	(-)-Spathulenol (C_{15})	0.10	-	-	-	-	0.10
44	20.44	Caryophyllene oxide (C_{15})	0.10	-	-	2.20	1.44	0.75
45	20.59	Viridiflorol (C_{15})	-	-	-	-	2.67	-
46	20.84	Ledol (C_{15})	-	-	-	-	1.00	0.21
47	21.73	δ-Cadinol (C_{15})	0.65	-	0.88	-	-	0.16
48	21.95	α-Cadinol (C_{15})	-	-	-	-	0.56	0.15
49	21.93	β-Eudesmol (C_{15})	1.05	-	-	-	-	0.28
50	22.01	γ-Eudesmol (C_{15})	1.81	-	-	-	-	-
51	22.57	α-Bisabolol (C_{15})	-	-	-	-	0.43	0.15
52	22.67	(*Z*)-Lanceol (C_{15})	-	-	-	-	0.39	-
53	24.85	β-Selinene (C_{15})	2.28	-	-	-	-	-
54	26.04	Hexahydrofarnesyl acetone	-	1.80	-	-	-	0.21
55	26.41	Diisobutyl phthalate	-	-	-	-	0.81	-
56	28.24	Butyl-octyl phthalate	-	-	-	-	0.53	0.56
57	30.11	Phytol (C_{20})	-	-	-	-	0.73	8.90
58	32.53	Heptacosane (alkane)	-	2.02	-	-	0.22	0.71
59	33.43	Octacosane (alkane)	-	1.04	-	-	0.20	0.68

* RT: retention time; TP—*Teucrium polium*; TH—*Teucrium hircanicum*; TB—*Teucrium botrys*; TCH—*Teucrium chamaedrys*; TF—*Teucrium flavum*; TO—*Teucrium orientale*; ** (C_{10})—monoterpenes; (C_{15})—sesquiterpenes; (C_{20})—diterpenes. Note: The plant material of *T. scordium* was not enough for essential oil extraction and antibacterial tests).

The chemical composition of the other species ranged from 13 constituents for the *T. hircanicum* (99.92%) essential oil to 15 for *T. botrys* (99.26%) and *T. chamaedrys* (98.92%), 29 for *T. orientale* (90.39%), and 30 for *T. flavum* (99.42%). The predominant components of *T. hircanicum* essential oil of Moldavian origin were α-himachalene (38.21%), β-farnesene (30.44%), and β-linalool (5.0%), and its composition differed from previously reported results [35]. Apart from the majority of compounds identified in *T. hircanicum* plants of Moldovan origin, Iranian scientists reported significant amounts of (*E*)-α-bergamotene (17.5–86.9%) and (*E*)-β-farnesene (0.5–21.4%), which differed quantitatively [36].

The *T. botrys* volatile oil differed from the other abovementioned species due to its high contents of β-caryophyllene (54.06%), alloaromadendrene (15.81%), and β-pinene (3.81%), and also differed from oil of the same species of Croatian origin which, except for β-caryophyllene (20.4%), contains α-humulene (13.9%) and β-farnesene (17.7%) as major constituents [22]. Compared to the essential oils of *T. chamaedrys* from Turkey or Croatia, which are characterized by increased contents of β-caryophyllene (26.9%) and germacrene D (22.8%) [22,37], the oil from Moldova was characterized by a higher content of β-caryophyllene (40.95%) together with germacrene D (22.09%) and α-caryophyllene (9.14%).

The main components of the essential oil of *T. flavum*—germacrene D (15.47%) and *a*-pinene (12.38%)—were found in most of the abovementioned species, but high content of β-bisabolene (29.96%) was characteristic only of this species. On the other hand, the essential oil from *T. flavum* of Moldovan origin, in terms of major components, differed substantially from that originating from Greece, in which the basic components are caryophyllene (13.5%) and caryophyllene oxide (8.5%) [19].

Surprisingly, one of the main components of the *T. orientale* essential oil was butylated hydroxytoluene (14.97%), found together with other main terpenic constituents such as α-caryophyllene (16.47%) and germacrene D (13.86%). In general, data on the chemical composition of *T. orientale* essential oil widely differ from country to country. [38,39]. Thus, Bagci et al. identified β-caryophyllene (15.3–19%), germacrene D (12.8–14.2%), and caryophyllene oxide (14.0–19.0%) as the main components of essential oil obtained from *T. orientale* var. *orientale*, and *T. orientale* var. *puberulens* collected in Turkey [39].

On the other hand, the essential oils from *T. orientale* subsp. *glabrescens* of Iranian origin showed high contents of β-cubebene (34.5%), α-cubebene (16.6%), α-copaene (10.1%), and β-caryophyllene (15.0%).

Summarizing the data of the GC–MS analysis, it is clearly visible that, depending on the major component, the analyzed species can be assigned to four chemo-types as follows: germacrene D (*T. polium*), α-himachalene (*T. hircanicum*), β-caryophyllene (*T. botrys*, *T. chamaedrys*, *T. orientale*), and β-bisabolene (*T. flavum*).

3.2. NAA Analysis of Elemental Content of Some Teucrium Species

The final data related to the contents of all 18 elements are reproduced in Table 2 together with the corresponding RP [40] values. It should be mentioned that some elements were detected only in certain species—such as Cr in *T. chamaedrys* and *T. botrys*, Ce in *T. polium*, and V and Ti in *T. flavum* and *T. botrys*—and were thus not included in Table 2.

The data presented in Table 2 show that the chemical composition of the analyzed plants was very different.

Table 2. Elemental content (mean of three measurements ± SD) of analyzed samples determined by NAA (in mg/kg), as well as reference plant values [32,40].

Element	*T. polium*	*T. hircanicum*	*T. chamaedrys*	*T. scordium*	*T. flavum*	*T. orientale*	*T. botrys*	RP [40]
Na	696 ± 55	195 ± 15	361 ± 25	1260 ± 7	388 ± 15	174 ± 7	715 ± 29	150
Mg	4350 ± 160	2430 ± 145	3260 ± 195	2670 ± 160	2350 ± 117	1700 ± 100	4270 ± 213	200
Al	5160 ± 64	336 ± 13	1810 ± 72	594 ± 24	1280 ± 64	490 ± 25	3090 ± 150	80
Cl	2580 ± 200	1850 ± 150	575 ± 45	9130 ± 730	538 ± 43	5420 ± 430	4130 ± 330	2000
K	18,700 ± 1100	17,700 ± 1060	14,900 ± 890	22,500 ± 1350	13,200 ± 1190	25,100 ± 2250	21,100 ± 1900	19,000
Ca	12,000 ± 1200	16,500 ± 1650	11,000 ± 1100	21,100 ± 2110	10,700 ± 1605	6610 ± 990	14,400 ± 2160	10,000
Sc	0.95 ± 0.05	0.063 ± 0.003	0.29 ± 0.01	0.145 ± 0.01	0.18 ± 0.007	0.081 ± 0.004	0.48 ± 0.01	0.02
Mn	132 ± 5	96 ± 3.8	74 ± 3	69 ± 2.9	54 ± 1.6	55.0 ± 1.7	97 ± 2.9	200
Fe	2520 ± 150	193 ± 19	760 ± 50	535 ± 40	473 ± 33	316 ± 22	1300 ± 78	150
Co	1.16 ± 0.08	0.48 ± 0.03	0.53 ± 0.03	0.265 ± 0.02	0.3 ± 0.02	0.48 ± 0.02	0.79 ± 0.04	0.2
Zn	34.4 ± 1.4	25.4 ± 1.1	20.2 ± 0.8	40 ± 1.6	30 ± 1.8	37.0 ± 2	50 ± 3	50
As	1.63 ± 0.06	0.94 ± 0.03	0.92 ± 0.03	1.34 ± 0.05	0.19 ± 0.01	0.091 ± 0.01	0.5 ± 0.05	0.1
Br	16.8 ± 0.7	11.3 ± 0.4	2.7 ± 0.1	8.5 ± 0.3	6.2 ± 0.2	21.3 ± 1	112 ± 4.5	4
Rb	22.5 ± 3.4	15.0 ± 2.2	9.4 ± 1.4	2.8 ± 0.9	5.8 ± 1.0	12.3 ± 2	13 ± 2.2	50
Sr	48.0 ± 4.8	36.0 ± 3.6	41 ± 4.1	118 ± 12	50 ± 4.0	26.0 ± 2.3	82 ± 6.6	50
Mo	0.43 ± 0.04	1.1 ± 0.1	0.41 ± 0.04	0.97 ± 0.09	n.d.	n.d.	n.d.	0.5
Sb	0.1 ± 0.007	0.037 ± 0.003	0.027 ± 0.002	0.039 ± 0.003	0.04 ± 0.004	0.043 ± 0.004	0.084 ± 0.007	0.1
Cs	0.4 ± 0.02	0.058 ± 0.003	0.12 ± 0.007	0.078 ± 0.005	0.08 ± 0.004	0.04 ± 0.004	0.21 ± 0.008	0.2
Ba	50.5 ± 4.5	7.9 ± 0.7	20.3 ± 1.8	28 ± 2.2	25 ± 1.5	7.7 ± 0.6	97 ± 4.8	40

In all samples, the most abundant element was K; its mass fraction ranged from 13,200 mg/kg in *T. flavum* to 25,100 mg/kg in *T. orientale*. Potassium (K) is one of the major elements required for plant growth and development (stomatal regulation and photosynthesis). Potassium participates in several biochemical processes related to protein synthesis, carbohydrate metabolism, and enzyme activation [41]. In analyzed plants, Ca was the second most abundant element; its lowest mass fraction (6610 mg/kg) was determined in *T. orientale*, while the highest (21,100 mg/kg) was in *T. scordium*. Calcium is an essential macroelement required for plants, playing a structural role as a counter-cation for inorganic and organic anions in the vacuole, and as an intracellular messenger in the cytosol [42]. In *T. polium*, *T. hircanicum*, *T. chamaedrys*, *T. scordium*, and *T. flavum*, the contents of K and Ca were almost at the same level. According to Zinicovscaia et al. [28], soil can be considered to be the main source of potassium and calcium in the studied plants. Moldova is characterized by abundant limestone soils. Magnesium (Mg) plays an important role in enzyme activities [43]. The range of content of Mg covered values from 1700 to 4350 mg/kg, while those of Cl ranged from 538 to 5420 mg/kg. Sodium content in the plants varied from 174 to 1260 mg/kg. For the majority of plants, Na is not an essential element [44].

Micronutrients such as Cr, Mn, Zn, Co, Mo, Br, and Fe are constitutive elements with specific functions in plant metabolism [45]. Among the microelements, Fe was the most abundant, as its concentration varied from 316 to 2520 mg/kg. Iron (Fe) is an essential microelement required for synthesis and other life processes of the cells [46]. Manganese (Mn) plays an important role in oxidation and reduction processes, and participates in the activation of more than 35 different enzymes [47]. The manganese content in the samples ranged from 55 μg/g to 132 μg/g, with the highest mass fraction in *T. polium*. Zinc levels in the analyzed plants ranged from 20 to 50 mg/kg. In plants, Zn acts as a functional, structural, or regulatory cofactor of a large number of enzymes [48]. The bromine content in samples varied between 2.7 and 172 mg/kg. According to Kataba-Pendias et al. [46], the natural Br content of plants should not exceed 40 ppm (approximately), and some higher values are related to pollution. Bromine content > 40 mg/kg was determined only in *T. botrys*. Molybdenum was determined in four of the analyzed species, and its content was 0.4–1.1 mg/kg.

Al, Sc, Ti, V, As, Rb, Sr, Sb, Cs, and Ba have no biological functions; their main sources in plants are soil (Al, Sc, Ti, V, Rb, Sr, Cs, and Ba) and anthropogenic activity (As, Sb). The content of As in the analyzed plants ranged from 0.09 to 1.6 mg/kg. Since the mass fraction

of As in plants grown on uncontaminated soils varies from 0.009 to 1.5 ppm [45], it can be concluded that the analyzed plants were grown on uncontaminated soil. The mass fraction of As did not exceed the limit of 1 mg/kg recommended for medicinal plants by the World Health Organization [49].

After careful examination, it could be remarked that there was a great discrepancy between the mass fractions of Mg, Cl, K, and Ca—belonging to the main groups of elements [40]—and the other elements, many of which were classified as enzymatic transition elements—such as Mn, Co, and Zn—or presumably contaminating ones, such as As and Br [40]. To all of them can additionally be included elements of which a particular role in plant metabolism has not yet been well evidenced, e.g., Na, Al, Rb, Sb, Cs, and Ba.

To attenuate the discrepancies that reached more than three orders of magnitude between the elements, all experimental mass fractions were normalized to the reference plant (RP) [40] and reproduced as a stacked bar chart, as shown in Figure 1. This procedure, which made the multitude of experimental mass fractions more compact, did not influence the subsequent PCA results, as PCA is based on the correlation and not on the statistical distances between data.

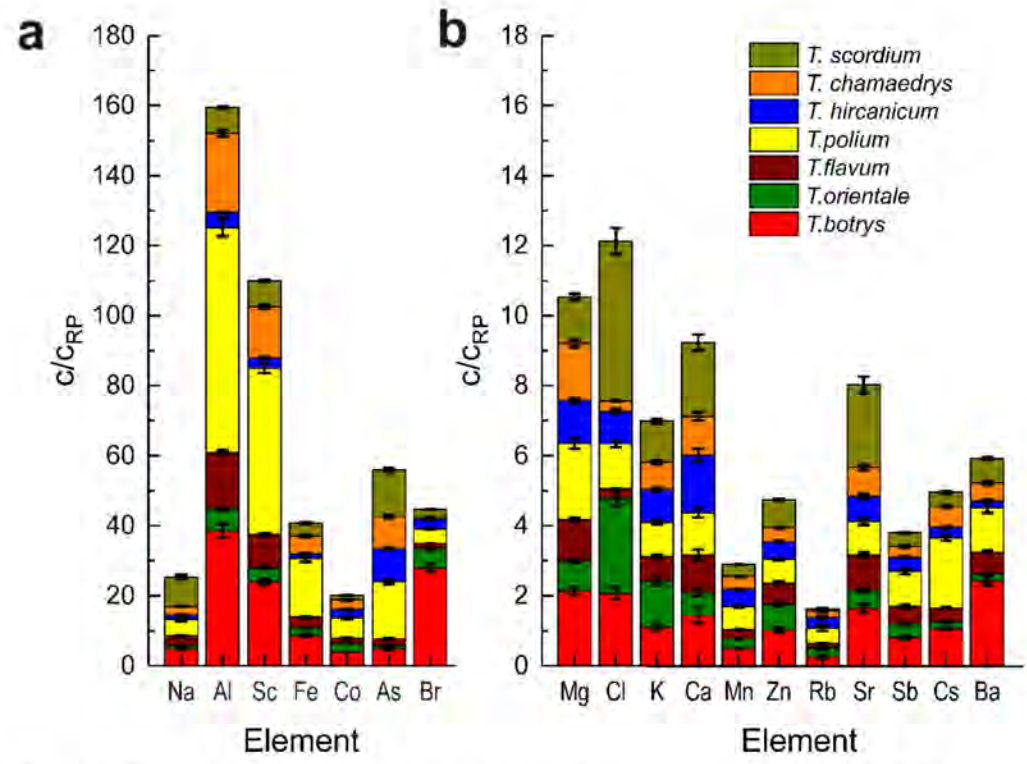

Figure 1. The stacked bar chart illustrates the distribution of mass fractions of all 18 elements determined in all analyzed plants; each taxon is normalized to the reference plant [39,40]: (**a**) elements with $C/C_{RP} > 20$; (**b**) elements with $C/C_{RP} < 20$.

Table 3 compares the mean values for the analyzed plants with the mean data obtained for *Teucrium* species collected in Bulgaria [50] and Serbia [51].

Table 3. Benchmarking of NAA analysis of some *Teucrium* spp. of Bulgarian [49,50], Serbian [50,51], and Moldavian origins (mass fraction in mg/kg).

Element	Present Study Range	*T. chamaedrys* [51]	*T. montanum* [51]	*T. chamaedrys* [50]	*T. polium* [50]	*T. montanum* [50]
Mg	2430–4350	1124	2826	1800	2031	3068
K	13,200–25,100	16,065	8267	-	-	-
Ca	6610–21,100	6557	3400	5668	7174	4856
Mn	54–132	46.7	42.3	26	40	46.4
Fe	193–2520	331	1266	725	735	898
Co	0.27–1.16	-	-	8.2	1.7	2.3
Zn	20–50	38	25	31	36	44
As	0.5–1.63	-	-	0.23	0.96	0.7

The mean mass fraction of Mg in the plants from Moldova was higher than the values reported for Serbia and Bulgaria, except for *T. montanum*. K, Ca, and Mn content in *Teucrium* species was the highest in comparison with literature data, while the content of Co was the lowest. Zn content was on the same level for all compared plants. Fe and As values were comparable with the data presented for Bulgaria, but higher than the values reported for Serbia.

3.3. Statistical Analysis

Both GC–MS and INAA provided valuable data concerning the presence of essential oils and trace elements in all *Teucrium* samples. Given the variability of the presence of essential oils, their presence could not be evidenced in all samples, as the data reproduced in Table 1 illustrate. Conversely, INAA was able to evidence the presence of 18 elements—essential and non-essential—in all samples (Table 2). Therefore, the completeness of INAA data permitted us to perform a pairwise ANOVA analysis only in the case of INAA data.

The reduced number of *Teucrium* species makes it difficult to establish the real type of distribution function of each element—normal or non-normal. Therefore, for a better analysis, we used both parametric and nonparametric tests, i.e., Tukey's Q and Mann–Whitney U tests, of which the probabilities of the mean and the distribution being equal, respectively, are reproduced in Table 4.

Table 4. The pairwise ANOVA parametric Tukey's Q test (**a**) and non-parametric Mann–Whitney U test (**b**) illustrating the probability that the means are equal (**a**) or the distributions are the same (**b**). The significant probabilities are presented with red ink.

a	*T. botrys*	*T. orientale*	*T. flavum*	*T. polium*	*T. hircanicum*	*T. chamaedrys*
T. orientale	0.493					
T. flavum	0.657	1.000				
T. polium	0.976	0.093	0.164			
T. hircanicum	0.518	1.000	1.000	0.102		
T. chamaedrys	0.882	0.995	1.000	0.359	0.996	
T. scordium	0.829	0.998	1.000	0.293	0.999	1.000
b						
T. orientale	0.038					
T. flavum	0.048	0.899				
T. polium	0.812	0.040	0.054			
T. hircanicum	0.048	0.704	0.937	0.064		
T. chamaedrys	0.085	0.800	0.912	0.071	0.962	
T. scordium	0.376	0.194	0.268	0.496	0.275	0.438

Upon careful analysis of the Table 4 data, it can be observed that the Mann–Whitney test proved to be more conservative—especially in the case of *T. scordium*, the mass fraction distribution of which appears quite different with respect to all other taxa—a peculiarity evidenced also on the Q-mode PCA (Figure 2).

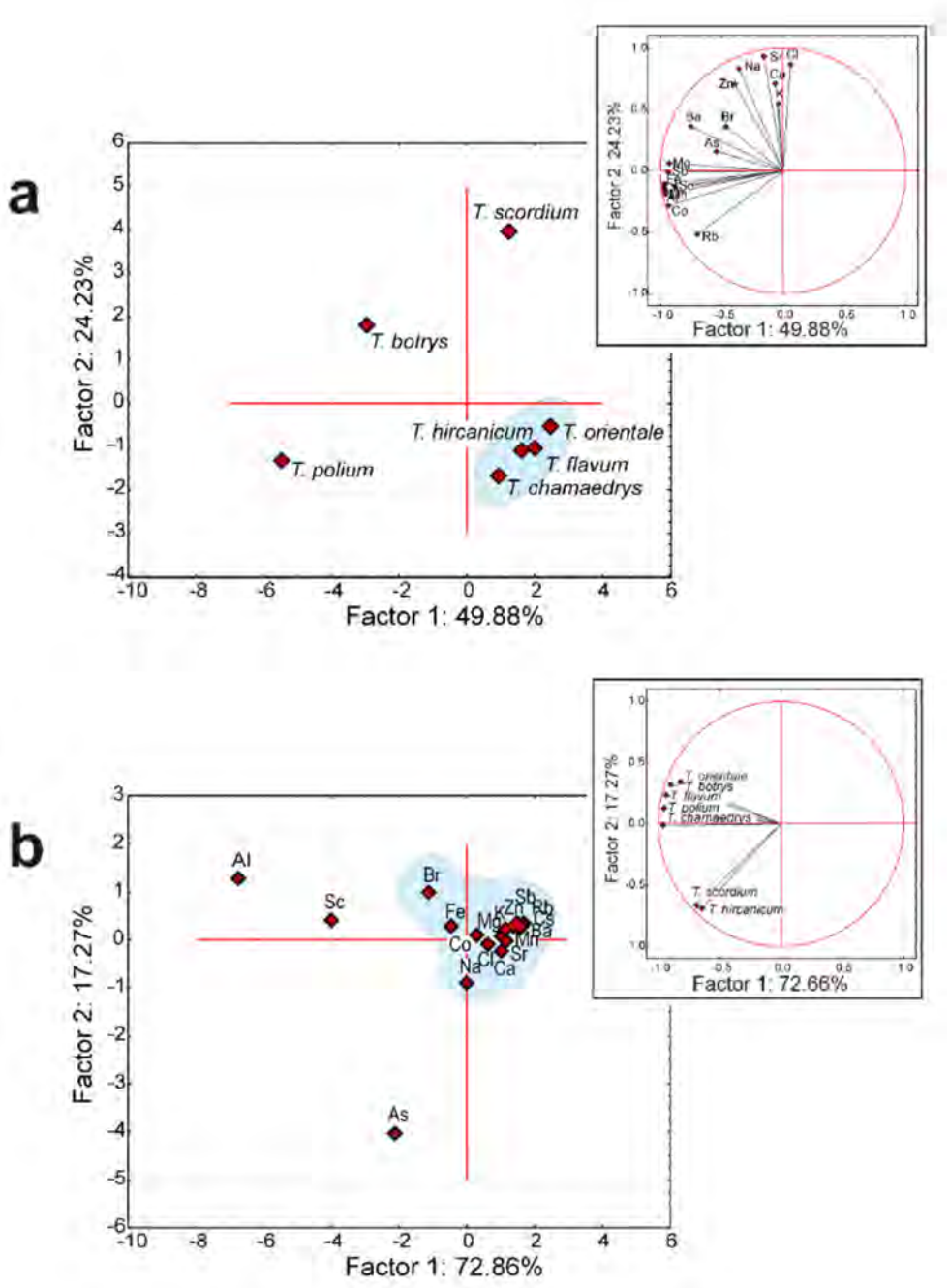

Figure 2. Biplots illustrating the results of Q (**a**)- and R (**b**)-mode PCA. The distribution of the first and second factor loadings is reproduced in the corresponding insets.

To go deeper into analysis, to evidence any existing reciprocal correlations between the investigated taxa or between the 18 elements, the results of the PCA in Q mode (7 taxa, the correlation of which is based on the mass fractions of the 18 elements) and R mode (elements correlated starting from their distribution in each taxon) are reproduced in Figure 2a,b, respectively.

This enabled us to determine the groups of items (taxa or elements) with the greatest contribution to the selection process [30]. This fact can be well understood by analyzing the factor (PC) loadings reproduced in the corresponding insets.

Accordingly, all *Teucrium* spp. formed four clusters; three of them—i.e., *T. polium*, *T. botrys*, and *T. scordium*—consisted of a single taxon, while the rest could be grouped in a single larger cluster (Figure 2a). This finding can be attributed to the presence of great amounts of Al, Sc, and As in *T. polium*, *T. botrys*, and *T. scordium* tissues. The distribution of the factor loading showed a dominance of Mg, Al, Sc, Mn, Fe, Co, Cs, and Sb in the first factor (PC1), while Na, Cl, K, Ca, Zn, and Sr seemed to be the main components of the second factor (PC2). The other elements—i.e., As, Br, Rb, and Ba—contributed to both factors. Given the great diversity of elements whose presence was evidenced in all taxa, we consider that in order to clarify the factor loading, it would be necessary to extend the present studies to more *Teucrium* species.

In the alternative R-mode PCA, the presence of three single-element clusters was observed—i.e., Al, Sc, and As—along with a fourth cluster containing the remaining 15 elements (Figure 2b). This finding can be well correlated with the anomalous presence of Al, Sc, and As in the abovementioned taxa (*T. polium*, *T. botrys*, and *T. scordium*). The corresponding factor loading distribution pointed towards a relatively equilibrated contribution of *T. scordium* and *T. hircanicum* to the first and second factors (Figure 2b, inset), while the other five taxa contributed mainly to the second factor or PC. The quasi-null contribution of *T. polium* and *T. botrys* in fact expressed the relatively complex dependence of the factors (PC) on the elemental composition of the considered taxon, where apart from Al, Sc, and As (the mass fractions of which were in some cases extremely high), the presence of the other elements most likely played a certain role.

The scree plots reproduced in Figure 3a,b illustrate the difference between the Q and R modes concerning the contribution of the first three factors (PC) to the analysis.

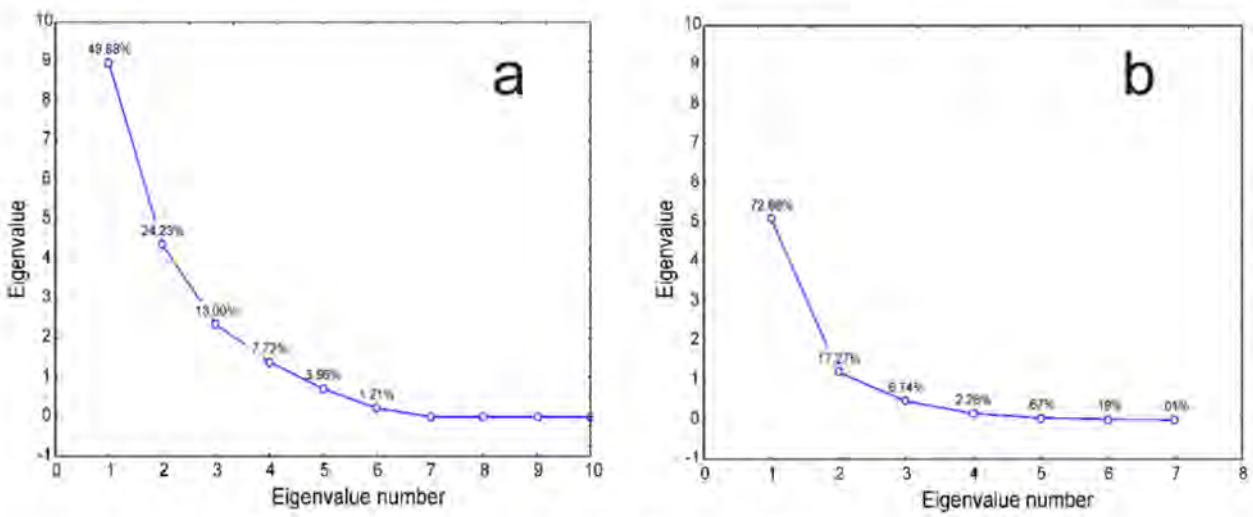

Figure 3. The scree plots of the Q (**a**)- and R (**b**)-mode PCA.

3.4. Antimicrobial Activity Assessment

The in vitro assessments of ethanolic extracts from *Teucrium* plants of Moldavian origin showed high antibacterial activity against both non-pathogenic Gram-positive/Gram-negative (*Bacillus subtilis* and *Pseudomonas fluorescens*) and phytopathogenic (*Xanthomonas campestris*, *Erwinia amylovora*, *Erwinia carotovora*) bacteria in the concentration range of 0.03–0.06%.

It is known that *Erwinia amylovora* is the causative agent of bacterial fire blight disease—a devastating plant disease that affects a wide range of *Rosaceae* species, and is a major global threat to the commercial production of apples and pears [52]. *Erwinia carotovora*

causes soft rot in potatoes, tomatoes, and cucumbers [53]. *Xanthomonas campestris* pv. *Vesication* causes bacterial spot in tomatoes (*Solanum lycopersicum* L.) and peppers (*Capsicum annuum*). Symptoms of infection include defoliation and chlorotic, necrotic lesions on leaves, stems, and flowers, which subsequently lead to a reduced yield of fruit [54].

The antifungal properties against *Candida utilis* strains lie in the concentrations in the range of 0.015–0.03% (Table 5).

Table 5. The antimicrobial activity of the ethanolic extracts from *Teucrium* plants.

Test-Microorganisms	Species, Double Successive Dilutions (Mbc, Mfc, Mg/Ml)					
	TP	**TH**	**TB**	**TCH**	**TF**	**TO**
Bacillus subtilis NCNM BB-01 (4.8 × 108 CFU/mL)	600	300	300	300	300	600
Pseudomonas fluorescens NCNM -PFB-01 (4.8 × 108 CFU/mL)	300	150	600	150	600	600
Xanthomonas campestris NCNM BX-01 (4.8 × 108 CFU/mL)	600	300	600	150	300	600
Erwinia amylovora NCNM BE-01 (4.8 × 108 CFU/mL)	600	150	600	300	150	150
Erwinia carotovora NCNM 177 BE-03 (4.8 × 108 CFU/mL)	300	150	600	300	300	600
Candida utilis NCNM Y-22 (3.0 × 107 CFU/mL)	300	150	150	300	300	300

MBC: minimal bactericidal concentration; MFC: minimal fungicidal concentration. TP—*Teucrium polium*; TH—*Teucrium hircanicum*; TB—*Teucrium botrys*, TCH—*Teucrium chamaedrys*; TF—*Teucrium flavum*; TO—*Teucrium orientale*.

The pronounced antimicrobial activity of the extracts can be explained by the significant content of flavonoids, and especially of sesquiterpene lactones. It is well known that this class of terpenoids is characterized by high antimicrobial activity [55,56]. The main mechanism of extract action includes the disruption of the cell membrane, which causes leakage of a considerable amount of cellular constituents, e.g., proteins and sugars; it can also cause a decrease in cellular weight, with significant changes in the permeability of the membrane, as confirmed by microscopy tests.

The antimicrobial activity of species of the Lamiaceae family has been presented in several studies. Thus, Askun et al. [57] showed considerable activity of *Thymus sipthorpii* Benth., *Satureja aintabensis* P.H. Davis, and *Micromeria juliana* (L.) Benth. ex Reich. against the four strains of *Mycobacterium tuberculosis*. Hydrolate of *Coridothymus capitatus* (L.) Reichenb. fil. showed good antimicrobial activity against bacteria and yeasts [31]. The essential oil of *Leucas inflata* Benth showed a powerful antibacterial effect against *Bacillus subtilis* and *Staphylococcus aureus* [30]. *Rosmarinus officinalis*, *Thymus capitatus*, *Origanum majorana*, and *Salvia officinalis* essential oils have shown good antibacterial activities against *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica*, *Bacillus subtilis*, and *Staphylococcus aureus* [34].

4. Conclusions

The results of the GC–MS analysis showed that the terpenic compounds constituted the main fraction of the studied volatile oils, with contents that ranged from 90.39% to 99.92%. The obtained data allowed us to conclude that the studied species belong to the following chemotypes: *Teucrium polium* (germacrene D), *T. hircanicum* (α-himachalene), *T. botrys*, *T. chamaedrys*, *T. orientale* (β-caryophyllene), and *T. flavum* (β-bisabolene). The mass fractions of 18 major, minor, and trace elements were determined by NAA in plants of 7 *Teucrium* species of Moldovan origin. The results highlighted differences in the elemental compositions of the investigated plants. The in vitro assessment of hydroalcoholic extracts from the analyzed *Teucrium* species confirmed their pronounced antibacterial and antifungal activity.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ATCC	American Type Culture Collection
CFU	Colony-forming unit
DNA	Deoxyribonucleic acid
GC–MS	Gas chromatography–mass spectrometry
MBC	Minimum bactericidal concentration
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
NAA	Neutron activation analysis
NCNM	National Collection of Non-Pathogenic Microorganisms
PCA	Principal component analysis
RNA	Ribonucleic acid
RP	Reference plants
RT	Retention time
SDA	Statistical data analysis

References

1. Cantino, P.D.; Harley, R.M.; Wagstaff, S.J. Genera of Labiatae: Status and classification. *Adv. Labiate Sci.* **1992**, *11*, 511–522.
2. Ernst, W.R.; Ernst, W.R. *Flora Europaea*; California Botanical Society: Berkeley, CA, USA, 1969; Volume 20.
3. Negru, A. *Determinatór de Plante din Flora Republicii Moldova*; Editura Universul: Chisinau, Moldova, 2007; ISBN 9789975470070.
4. Rizk, A.M.; Hammouda, F.M.; Rimpler, H.; Kamel, A. Iridoids and flavonoids of *Teucrium polium* herb. *Planta Med.* **1986**, *52*, 87–88. [CrossRef]
5. Suleiman, M.S.; Abdul-Ghani, A.S.; Al-Khalil, S.; Amin, R. Effect of *Teucrium polium* boiled leaf extract on intestinal motility and blood pressure. *J. Ethnopharmacol.* **1988**, *22*, 111–116. [CrossRef]
6. Bruno, M.; Maggio, A.M.; Piozzi, F.; Puech, S.; Rosselli, S.; Simmonds, M.S.J. Neoclerodane diterpenoids from *Teucrium polium* subsp. polium and their antifeedant activity. *Biochem. Syst. Ecol.* **2003**, *31*, 1051–1056. [CrossRef]
7. Frezza, C.; Venditti, A.; Matrone, G.; Serafini, I.; Foddai, S.; Bianco, A.; Serafini, M. Iridoid glycosides and polyphenolic compounds from *Teucrium chamaedrys* L. *Nat. Prod. Res.* **2018**, *32*, 1583–1589. [CrossRef]
8. Grubešić, R.J.; Kremer, D.; Vladimir-Knežević, S.; Rodríguez, J.V. Analysis of polyphenols, phytosterols, and bitter principles in *Teucrium* L. species. *Cent. Eur. J. Biol.* **2012**, *7*, 542–550. [CrossRef]
9. Esmaeili, M.A.; Yazdanparast, R. Hypoglycaemic effect of *Teucrium polium*: Studies with rat pancreatic islets. *J. Ethnopharmacol.* **2004**, *95*, 27–30. [CrossRef]
10. Rasekh, H.R.; Khoshnood-Mansourkhani, M.J.; Kamalinejad, M. Hypolipidemic effects of *Teucrium polium* in rats. *Fitoterapia* **2001**, *72*, 937–939. [CrossRef]

11. Parsaee, H.; Shafiee-Nick, R. Anti-spasmodic and anti-nociceptive effects of *Teucrium polium* aqueous extract. *Iran. Biomed. J.* **2006**, *10*, 145–149.
12. Qabaha, K.; Hijawi, T.; Mahamid, A.; Mansour, H.; Naeem, A.; Abbadi, J.; Al-Rimawi, F. Anti-inflammatory and antioxidant activities of *Teucrium polium* leaf extract and its phenolic and flavonoids content. *Asian J. Chem.* **2021**, *33*, 881–884. [CrossRef]
13. Bello, R.; Barrachina, M.D.; Martínez-Cuesta, M.A.; Esplugues, J.; Yúfera, E.P. Evaluation of the acute toxicity, analgesic and CNS activities of different species of *Teucrium* genus. *Phyther. Res.* **1995**, *9*, 277–280. [CrossRef]
14. Raei, F.; Ashoori, N.; Eftekhar, F.; Yousefzadi, M. Chemical composition and antibacterial activity of *Teucrium polium* essential oil against urinary isolates of *Klebsiella pneumoniae*. *J. Essent. Oil Res.* **2014**, *26*, 65–69. [CrossRef]
15. Stanković, M.S.; Mitrović, T.L.J.; Matić, I.Z.; Topuzović, M.D.; Stamenković, S.M. New values of *Teucrium* species: In Vitro study of cytotoxic activities of secondary metabolites. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2015**, *43*, 41–46. [CrossRef]
16. Tatjana, K.; Marina, M.; Aleksandra, T.; Tatjana, S.; Zorica, J.; Branislava, L. Cytotoxicity and antimicrobial activity of *Teucrium scordium* L. (*Lamiaceae*) extracts. *Afr. J. Microbiol. Res.* **2011**, *5*, 2950–2954. [CrossRef]
17. Stankovic, M.S.; Curcic, M.G.; Zizic, J.B.; Topuzovic, M.D.; Solujic, S.R.; Markovic, S.D. Teucrium plant species as natural sources of novel anticancer compounds: Antiproliferative, proapoptotic and antioxidant properties. *Int. J. Mol. Sci.* **2011**, *12*, 4190–4205. [CrossRef]
18. Javidnia, K.; Miri, R. Composition of the essential oil of *Teucrium orientate* L. ssp. orientate from Iran. *J. Essent. Oil Res.* **2003**, *15*, 118–119. [CrossRef]
19. Formisano, C.; Rigano, D.; Senatore, F.; Bruno, M.; Maggio, A.; Piozzi, F. Chemical composition of the essential oil of *Teucrium flavum* ssp. flavum from Zakynthos, Greece. *Rec. Nat. Prod.* **2012**, *6*, 306–310.
20. Presti, M.L.; Crupi, M.L.; Costa, R.; Dugo, G.; Mondello, L.; Ragusa, S.; Santi, L. Seasonal variations of *Teucrium flavum* L. essential oil. *J. Essent. Oil Res.* **2010**, *22*, 211–216. [CrossRef]
21. Bezić, N.; Vuko, E.; Dunkić, V.; Ruščić, M.; Blažević, I.; Burčul, F. Antiphytoviral activity of sesquiterpene-rich essential oils from four croatian *Teucrium* species. *Molecules* **2011**, *16*, 8119–8129. [CrossRef]
22. Kovacevic, N.N.; Lakusic, B.S.; Ristic, M.S. Composition of the essential oils of seven *Teucrium* species from serbia and montenegro. *J. Essent. Oil Res.* **2001**, *13*, 163–165. [CrossRef]
23. Lograda, T.; Ramdani, M.; Chalard, P.; Figueredo, G.; Deghar, A. Chemical analysis and antimicrobial activity of *Teucrium polium* L. essential oil from Western Algeria. *J. Med. Plants Res.* **2013**, *7*, 897–902. [CrossRef]
24. Hammami, S.; El Mokni, R.; Faidi, K.; Falconieri, D.; Piras, A.; Procedda, S.; Mighri, Z.; El Aouni, M.H. Chemical composition and antioxidant activity of essential oil from aerial parts of *Teucrium flavum* L. subsp. flavum growing spontaneously in Tunisia. *Nat. Prod. Res.* **2015**, *29*, 2336–2340. [CrossRef] [PubMed]
25. El Atki, Y.; Aouam, I.; El Kamari, F.; Taroq, A.; Lyoussi, B.; Oumokhtar, B.; Abdellaoui, A. Phytochemistry, antioxidant and antibacterial activities of two Moroccan *Teucrium polium* L. subspecies: Preventive approach against nosocomial infections. *Arab. J. Chem.* **2020**, *13*, 3866–3874. [CrossRef]
26. Mahmoudi, R.; Zare, P.; Hassanzadeh, P.; Nosratpour, S. Effect of *Teucrium polium* essential oil on the physicochemical and sensory properties of probiotic yoghurt. *J. Food Process. Preserv.* **2014**, *38*, 880–888. [CrossRef]
27. Ciocarlan, A.; Lupascu, L.; Aricu, A.; Dragalin, I.; Ciocarlan, N.; Zinicovscaia, I.; Slanina, V.; Yushin, N. Chemical Composition of the Essential Oil and Antimicrobial Properties of Crude Extract from *Tanacetum corymbosum* (L.) Shi. Bip. *Chem. J. Mold.* **2021**, *16*, 83–90. [CrossRef]
28. Zinicovscaia, I.; Duliu, O.G.; Culicov, O.A.; Frontasyeva, M.V.; Sturza, R. Major and trace elements distribution in moldavian soils. *Rom. Rep. Phys.* **2018**, *70*, 701.
29. Zinicovscaia, I.; Duliu, O.G.; Culicov, O.A.; Sturza, R.; Bilici, C.; Gundorina, S. Geographical Origin Identification of Moldavian Wines by Neutron Activation Analysis. *Food Anal. Methods* **2017**, *10*, 3523–3530. [CrossRef]
30. Mothana, R.A.; Noman, O.M.; Al-Sheddi, E.S.; Khaled, J.M.; Al-Said, M.S.; Al-Rehaily, A.J. Chemical composition, in vitro antimicrobial, free-radical-scavenging and antioxidant activities of the essential oil of Leucas inflata Benth. *Molecules* **2017**, *22*, 367. [CrossRef]
31. Marino, A.; Nostro, A.; Mandras, N.; Roana, J.; Ginestra, G.; Miceli, N.; Taviano, M.F.; Gelmini, F.; Beretta, G.; Tullio, V. Evaluation of antimicrobial activity of the hydrolate of *Coridothymus capitatus* (L.) Reichenb. Fil. (*Lamiaceae*) alone and in combination with antimicrobial agents. *BMC Complement. Med. Ther.* **2020**, *20*, 89. [CrossRef]
32. Jollife, I.T.; Cadima, J. Principal component analysis: A review and recent developments. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* **2016**, *374*, 20150202. [CrossRef]
33. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. Past: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* **2001**, *4*, 178.
34. Moumni, S.; Elaissi, A.; Trabelsi, A.; Merghni, A.; Chraief, I.; Jelassi, B.; Chemli, R.; Ferchichi, S. Correlation between chemical composition and antibacterial activity of some lamiaceae species essential oils from Tunisia. *BMC Complement. Med. Ther.* **2020**, *20*, 103. [CrossRef] [PubMed]
35. Jaradat, N.A. Review of the taxonomy, ethnobotany, phytochemistry, phytotherapy and phytotoxicity of germander plant (*Teucrium polium* L.). *Asian J. Pharm. Clin. Res.* **2015**, *8*, 13–19.
36. Rahimi, M.A.; Nazeri, V.; Andi, S.A.; Sefidkon, F. Variation in essential oil composition of *Teucrium hircanicum* L. from Iran-A rich source of (E)-α-bergamotene. *Nat. Prod. Res.* **2019**, *33*, 1227–1232. [CrossRef]

37. Bagci, E.; Yazgin, A.; Hayta, S.; Cakilcioglu, U. Composition of the essential Oil of *Teucrium chamaedrys* L. (*Lamiaceae*) from Turkey. *J. Med. Plants Res.* **2010**, *4*, 2588–2590.
38. Aberumand, M.; Asgarpanah, J. Essential Oil Composition of *Teucrium orientale* subsp. glabrescens from Iran. *Chem. Nat. Compd.* **2017**, *53*, 381–382. [CrossRef]
39. Kucukbay, F.Z.; Yildiz, B.; Kuyumcu, E.; Gunal, S. Chemical composition and antimicrobial activities of the essential oils of *Teucrium orientale* var. orientale and *Teucrium orientale* var. puberulens. *Chem. Nat. Compd.* **2011**, *47*, 833–836. [CrossRef]
40. Markert, B. Establishing of "Reference Plant" for inorganic characterization of different plant species by chemical fingerprinting. *Water Air Soil Pollut.* **1992**, *64*, 533–538. [CrossRef]
41. Hasanuzzaman, M.; Bhuyan, M.H.M.B.; Nahar, K.; Hossain, M.S.; Al Mahmud, J.; Hossen, M.S.; Masud, A.A.C.; Moumita; Fujita, M. Potassium: A vital regulator of plant responses and tolerance to abiotic stresses. *Agronomy* **2018**, *8*, 31. [CrossRef]
42. White, P.J.; Broadley, M.R. Calcium in plants. *Ann. Bot.* **2003**, *92*, 487–511. [CrossRef]
43. Guo, W.; Nazim, H.; Liang, Z.; Yang, D. Magnesium deficiency in plants: An urgent problem. *Crop. J.* **2016**, *4*, 83–91. [CrossRef]
44. Maathuis, F.J.M. Sodium in plants: Perception, signalling, and regulation of sodium fluxes. *J. Exp. Bot.* **2014**, *65*, 849–858. [CrossRef]
45. Chizzola, R.; Michitsch, H.; Franz, C. Monitoring of metallic micronutrients and heavy metals in herbs, spices and medicinal plants from Austria. *Eur. Food Res. Technol.* **2003**, *216*, 407–411. [CrossRef]
46. Kabata-Pendias, A. *Trace Elements in Soils and Plants*; CRC Press: Boca Raton, FL, USA, 2000.
47. Mousavi, S.R.; Shahsavari, M.; Rezaei, M. A general overview on manganese (Mn) importance for crops production. *Aust. J. Basic Appl. Sci.* **2011**, *5*, 1799–1803.
48. Brown, P.H.; Cakmak, I.; Zhang, Q. Form and Function of Zinc Plants. In *Zinc in Soils and Plants*; Springer: Dordrecht, The Netherlands, 1993; pp. 93–106.
49. World Health Organization. Pharmaceuticals Unit Quality control methods for medicinal plant materials. *Geneva World Health Organ.* **1998**, *122*, 1–84.
50. Pavlova, D.; Karadjova, I. Chemical analysis of *Teucrium* species (*Lamiaceae*) growing on serpentine soils in Bulgaria. *J. Plant Nutr. Soil Sci.* **2012**, *175*, 891–899. [CrossRef]
51. Zlatić, N.M.; Stanković, M.S.; Simić, Z.S. Secondary metabolites and metal content dynamics in *Teucrium montanum* L. and *Teucrium chamaedrys* L. from habitats with serpentine and calcareous substrate. *Environ. Monit. Assess.* **2017**, *189*, 110. [CrossRef]
52. Piqué, N.; Miñana-Galbis, D.; Merino, S.; Tomás, J.M. Virulence factors of *Erwinia amylovora*: A review. *Int. J. Mol. Sci.* **2015**, *16*, 12836–12854. [CrossRef]
53. Benada, M.; Boumaaza, B.; Boudalia, S.; Khaladi, O.; Guessas, B. Variability of Aggressiveness and Virulence of *Erwinia carotovora* subsp. Carotovorum Causing the Soft Rot on Potato Tubers in the Western of Algeria. *Int. J. Plant Biol.* **2018**, *9*, 7568. [CrossRef]
54. Tamir-Ariel, D.; Navon, N.; Burdman, S. Identification of genes in *Xanthomonas campestris* pv. *vesicatoria* induced during its interaction with tomato. *J. Bacteriol.* **2007**, *189*, 6359–6371. [CrossRef]
55. Chadwick, M.; Trewin, H.; Gawthrop, F.; Wagstaff, C. Sesquiterpenoids lactones: Benefits to plants and people. *Int. J. Mol. Sci.* **2013**, *14*, 12780–12805. [CrossRef] [PubMed]
56. Jansen, B.J.M.; De Groot, A. The occurrence and biological activity of drimane sesquiterpenoids. *Nat. Prod. Rep.* **1991**, *8*, 309–318. [CrossRef] [PubMed]
57. Askun, T.; Tekwu, E.M.; Satil, F.; Modanlioglu, S.; Aydeniz, H. Preliminary antimycobacterial study on selected Turkish plants (*Lamiaceae*) against *Mycobacterium tuberculosis* and search for some phenolic constituents. *BMC Complement. Altern. Med.* **2013**, *13*, 365. [CrossRef] [PubMed]