Article

Not peer-reviewed version

Chemical Composition of Essential Oil and Evaluation of Antimicrobial and Antidiabetic Activities of Teucrium kotschyanum Poech (Lamiaceae)

Betül Demirci , Nur Tan , Çağlayan Ünsal Gürer * , Seçil Yazıcı Tütüniş , Gizem Gülsoy-Toplan , Gökalp İşcan

Posted Date: 12 March 2025

doi: 10.20944/preprints202503.0806.v1

Keywords: Teucrium kotschyanum; Lamiaceae; essential oil; GC-MS; antimicrobial activity; antidiabetic activity

Article

Chemical Composition of Essential Oil and Evaluation of Antimicrobial and Antidiabetic Activities of *Teucrium kotschyanum* Poech (Lamiaceae)

Çağlayan Ünsal Gürer [1,*], Seçil Yazıcı Tütüniş [1], Gizem Gülsoy-Toplan [2], Gökalp İşcan [3], Betül Demirci [3] and Nur Tan [1]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Istanbul University, 34116, Beyazıt, Istanbul, Türkiye; secilyaz@istanbul.edu.tr (S.Y.T.); nurtan@istanbul.edu.tr (N.T.)
[2] Department of Pharmacognosy, Hamidiye Faculty of Pharmacy, University of Health Sciences, 34668, Üsküdar, Istanbul, Türkiye; gizem.gulsoytoplan@sbu.edu.tr
[3] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskişehir, Türkiye; giscan@anadolu.edu.tr (G.İ.); bdemirca@anadolu.edu.tr (B.D.)
* Correspondence: cunsal@istanbul.edu.tr

Abstract: The genus *Teucrium* L., member of Lamiaceae, is represented by approximately 36 species in Türkiye. In this study, the essential oil composition of the aerial parts of *Teucrium kotschyanum* Poech collected from İzmir (Ödemiş) was investigated by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The major compounds were identified as germacrene D (20.0 %), α-cadinol (6.9 %), β-bourbonene (6.6 %), hexadecanoic acid (6.6 %), δ-cadinene (5.9 %) and manool (4.1 %). The antibacterial and antifungal activities of the essential oil were studied against six bacteria and six fungi using the microdilution broth method. The essential oil showed moderate to weak inhibitory effects. The minimum inhibitory concentration (MIC) ranged from 125 to 2000 μg/mL for all tested microorganisms. The present study was also designed to evaluate the antidiabetic effects of the essential oil and extracts of the root and aerial parts of Teucrium kotschyanum via their potential inhibition of the related key enzymes. Essential oil showed significantly higher activity with an IC50 value of 100.25 mg/mL, than the positive control, with an IC50 value of 122.03 mg/mL. Among the extracts, the best activity was observed in the petroleum ether and ethyl acetate extracts of root parts, with the inhibition value of 94.79%, 55.49%, respectively.

Keywords: *Teucrium kotschyanum*; Lamiaceae; essential oil; GC-MS; antimicrobial activity; antidiabetic activity

1. Introduction

Türkiye is an important gene center in the Lamiaceae family. Lamiaceae plants are rich in secondary metabolites and can be extensively used in traditional medicine to treat various disorders [1]. In Türkiye, the genus *Teucrium* L., member of Lamiaceae, is represented by 36 species (48 taxa), 17 of which are endemic [2–11]. *Teucrium* species have been used as medicinal herbs for over 2000 years. They have antioxidant, antimicrobial and anti-inflammatory properties [12,13] and are commonly used in folk medicine as a treatment for diabetes, gastric ulcer, and intestinal inflammation, as well as a diuretic, antiseptic, and anti-helminthic [13,14]. In Greek folk medicine, infusion of the aerial parts of *Teucrium flavum* L. has been used orally as an antidiabetic and externally as an astringent to heal skin eruptions and wounds [15]. *Teucrium chamaedrys* L. and *T. polium* L. are widely distributed in Türkiye and have many medicinal uses in traditional medicine. Infusions prepared from the aerial parts of *T. chamaedrys* subsp. *chamaedrys* are consumed as tea for ulcers in the mouth and for kidney infections in the Bilecik region of Türkiye, whereas the aerial parts of T. polium are used as appetizers and karminatives for pain in the stomach and for wound healing [16]. *T. polium* infusions are also used for stomachache and as an analgesic in the Aydıncık district of Mersin [17].

Phytochemical studies have reported that *Teucrium* L. species are rich sources of diterpenoids and contain monoterpenes, sesquiterpenes, triterpenes, iridoids, flavonoids, and essential oils [18].

 doi:10.20944/preprints202503.0806.v1

As well as the genus *Teucrium* is one of the richest sources of neoclerodane diterpenes the aerial parts of *Teucrium* species were well-known to produce essential oils and essential oils of *Teucrium* species have been the subject of many studies. Many species of this genus are used in the food industry and show antimicrobial, antioxidant, and antifungal activities, rendering them useful as natural preservatives [19].

Teucrium species in Türkiye are classified into eight sections in the Flora of Turkey [20] and *T. kotschyanum* Poech is a member of sect. Scorodonia (Hill.) Schreber. It is a perennial herb that is rhizomatous and up to 80 cm in height. The flowers are yellow-green and the flowering season is between May and July. This species is rarely used in traditional medicine as an antipyretic or antidiabetic agent [21]. From the aerial parts of *T. kotschyanum* three neo-clerodane diterpenoids (12-epiteucvidin, 12-epiteuflin, and teukotschyn) were isolated, and ursolic acid, flavones cirsimaritin and cirsiliol, and six previously known neo-clerodane diterpenoids were also found [22]. A comparative study has been carried out on the essential oils of *T. cyprium* ssp. *cyprium*, *T. micropodioides*, *T. divaricatum* ssp. *canescens* and *T. kotschyanum* which are growing in Cyprus, and caryophyllene, humulene, cubebene, and burbonene were found to be present in high percentages mounting to 60% of the total *T. kotschyanum* essential oil [23].

Teucrium species are known for their medicinal properties and exhibit various biological activities, including broad-spectrum antimicrobial activity [24]. The studied species have been found to be more active against gram-positive bacteria than gram-negative bacteria and fungi. The essential oils were more potent than the extracts. The essential oils from *T. orientale*, *T. africanum*, *T. ramosissimum*, *T. mascatence*, *T. yemense*, *T. massiliense*, *T. scordonia* have been investigated for their antimicrobial activity, and *Teucrium polium* is one of the most tested *Teucrium* species, which exhibits pronounced activity [24].

Teucrium species also exhibit antihyperglycemic activity and reduce blood glucose levels [24–28].

In this study, the essential oil composition of the aerial parts of *Teucrium kotschyanum* Poech collected from Türkiye (Ödemiş-İzmir) at the flowering stage was investigated using gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The antibacterial and antifungal activities of the essential oils were examined against six bacteria and six fungi using microdilution broth method. Additionally, the antidiabetic activity of the essential oil and various extracts (petroleum ether, ethyl acetate, and methanol) of the species were evaluated.

2. Results

2.1. Chemical Composition of Essential Oil

In this study, we aimed to investigate the composition of essential oil obtained from the aerial parts of *Teucrium kotschyanum* Poech collected in İzmir (Ödemiş)-Türkiye at the flowering stage using GC and GC-MS. The yield of essential oil from the aerial parts of the plant was found to be 0.4%.

According to the *Teucrium* species studied and their plant origin, essential oil yields ranged from 0.05% to 1.5%, and the amounts of main constituents (mono- and sesquiterpene hydrocarbons and oxygenated sesquiterpenes) differed notably [29,30]. The essential oil composition of the aerial parts of the *Teucrium kotschyanum* was examined in our study, and 53 constituents represented 96.2% of the total components in the oil have been identified. The retention indices and percentage compositions are listed in Table 1. The major compounds were identified as germacrene D (20.0%), α-cadinol (6.9%), β-bourbonene (6.6%), hexadecanoic acid (6.6%), δ-cadinene (5.9%), manool (4.1%), other significant compounds were found as alloaromadendrene (3.0%), linalool (2.8%), T-muurolol (2.8%), viridiflorol (2.7%), T-cadinol (2.5%), and β-caryophyllene (2.0%).

Table 1. The composition of the essential oil of *Teucrium kotschyanum* Poech.

Compound	RRI	%	IM
β-Pinene	1118	0.4	t_R, MS
Sabinene	1132	0.5	t_R, MS
Myrcene	1174	0.1	t_R, MS
Limonene	1203	1.2	t_R, MS
γ-Terpinene	1255	0.2	t_R, MS
p-Cymene	1280	0.3	t_R, MS

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 March 2025 doi:10.20944/preprints202503.0806.v1

Terpinolene	1290	0.2	t_R, MS
1-Octen-3-ol	1452	1.0	MS
α-Cubebene	1466	0.1	MS
α-Copaene	1497	1.2	MS
α-Bourbonene	1528	0.5	MS
β-Bourbonene	1535	**6.6**	MS
Linalool	1553	2.8	t_R, MS
β-Ylangene	1589	0.1	MS
β-Copaene	1597	0.2	MS
Terpinen-4-ol	1611	0.9	t_R, MS
β-Caryophyllene	1612	**2.0**	t_R, MS
Alloaromadendrene	1661	3.0	MS
epi-Zonarene	1677	0.1	MS
α-Humulene	1687	2.2	t_R, MS
γ-Muurolene	1704	1.2	MS
α-Terpineol	1706	1.1	t_R, MS
Bicyclosesquiphellandrene	1722	1.3	MS
Germacrene D	1726	**20.0**	MS
α-Muurolene	1740	2.3	MS
δ-Cadinene	1773	5.9	MS
γ-Cadinene	1776	3.7	MS
Methyl salicylate	1798	0.3	t_R, MS
α-Cadinene	1807	0.4	MS
(*E*)-β-Damascenone	1838	0.6	MS
Calamenene	1849	0.7	MS
epi-Cubebol	1900	0.4	MS
α-Calacorene	1941	0.1	MS
Cubebol	1957	0.7	MS
1-*endo*-Bourbonanol	1968	0.1	MS
γ-Calacorene	1984	0.1	MS
Caryophyllene oxide	2008	0.8	t_R, MS
Salvial-4(14)-en-1-one	2037	0.8	MS
(*E*)-Nerolidol	2050	0.2	MS
Humulene epoxide-II	2071	0.9	MS
Cubenol	2080	0.6	MS
1-*epi*-Cubenol	2088	0.7	MS
Viridiflorol	2104	2.7	MS
Salviadienol	2130	0.7	MS
Hexahydrofarnesyl acetone	2131	0.9	MS
Spathulenol	2144	0.2	MS
T-Cadinol	2187	**2.5**	MS
T-Muurolol	2209	**2.8**	MS
δ-Cadinol (=*Torreyol*)	2219	1.0	MS
α-Cadinol	2255	**6.9**	MS
Dodecanoic acid	2503	1.3	t_R, MS
Manool	2679	4.1	MS
Hexadecanoic acid	2931	6.6	t_R, MS
Total		**96.2**	

RRI: Relative retention indices calculated against *n*-alkanes; %: calculated from FID data; tr : Trace (< 0.1 %); IM: Identification method; t_R: identification based on the retention times of genuine compounds on the HP Innowax column; MS: identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

 doi:10.20944/preprints202503.0806.v1

2.2. Antimicrobial Activity

The antimicrobial activity of the essential oil against Gram-positive and Gram-negative bacteria, as well as six fungal strains, was tested by broth microdilution methods. The essential oil from the aerial parts of *T. kotschyanum* showed moderate to weak inhibitory effects compared to standard antimicrobials. The minimum inhibitory concentration (MIC) ranged from 5 to 2000 μg/mL for all tested microorganisms. The results of the antimicrobial activities of the essential oil are given in Table 2.

Table 2. Minimum inhibitory concentrations (μg/mL) of essential oil of *Teucrium kotschyanum.*

	Microorganisms											
	Gram (+) bacteria			Gram (-) bacteria			Fungi					
EO / Reference standard	*Sa*	*Se*	*Bc*	*Bs*	*Pa*	*St*	*Ca*	*Cg*	*Ck*	*Cp*	*Ct*	*Cu*
Essential oil	500	2000	250	500	1000	2000	1000	1000	1000	500	1000	125
Ampicillin-Na	16	0.5	1	1	32	0.5	-	-	-	-	-	-
Chloramphenicol	1	2	4	1	64	2	-	-	-	-	-	-
Amphotericin	-	-	-	-	-	-	1	1	1	0.5	1	1
Ketoconazole	-	-	-	-	-	-	0.06	0.06	1	0.06	0.06	0.12

Sa: Staphylococcus aureus, Se: Staphylococcus epidermidis, Bc: Bacillus cereus, Bs: Bacillus subtilis, Pa: Pseudomonas aeruginosa, St: Salmonella typhimurium, Ca: Candida albicans, Cg: Candida glabrata, Ck: Candida krusei, Cp: Candida parapsilosis, Ct: Candida tropicalis, Cu: Candida utilis,-: not tested.

2.3. α-Glucosidase Inhibition Activity

The present study was also conducted to evaluate the antidiabetic effect of essential oil and extracts of the root and aerial parts of *Teucrium kotschyanum* via their inhibitory potential on the related key enzymes. Essential oil showed significantly higher activity with an IC_{50} value of 100.25 mg/mL, than the positive control, with an IC_{50} value of 122.03 mg/mL. Among the extracts, the best activity was observed in the petroleum ether and ethyl acetate extracts of root parts, with the inhibition value of 94.79%, 55.49%, respectively.

2.3.1. % Inhibition of Teucrium Kotschyanum Extracts

The inhibition (%) of *T. kotschyanum* extracts is presented in Table 3 and Figure 1. Among tested six plant extracts, petroleum ether extract of roots (RO-PE) were the most active, followed by ethyl acetate extract of roots (RO-EA) at 125 μg/mL concentration. These extracts exhibited a higher α-glucosidase inhibitory activity than acarbose at the same concentration (125 μg/mL). Aerial parts extracts of petroleum ether (AP-PE) and ethyl acetate (AP-EA) showed lower activity while methanol extracts of aerial parts (AP-MeOH) and roots (RO-MeOH) showed no significant difference with acarbose in the point of α-glucosidase inhibition at 125 μg/mL.

Table 3. α-glucosidase inhibitory activity of T. kotschyanum extracts and acarbose (positive control) at 125 μg/mL.

Sample	**Inhibition% of α-glucosidase**
AP-PE	24.63 ± 2.12^{a}
RO-PE	94.79 ± 1.10^{b}
AP-EA	29.74 ± 1.90^{a}
RO-EA	55.49 ± 2.83^{c}
AP-MeOH	40.54 ± 0.96^{d}
RO-MeOH	38.97 ± 0.67^{d}
Acarbose	42.8 ± 2.50^{d}

Values are expressed as mean ± SEM (n=4). The values with different superscripts (a-d) shows significant difference ($p < 0.05$).

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 March 2025 doi:10.20944/preprints202503.0806.v1

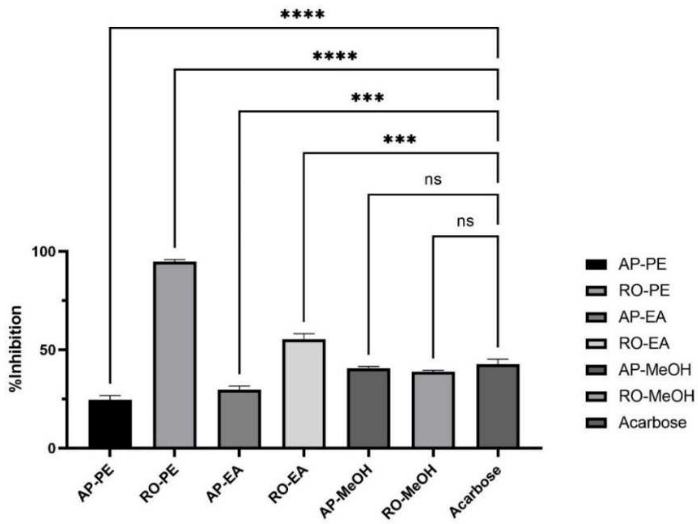

Figure 1. Comparison of α-glucosidase inhibitory activity (%) of T. kotschyanum extracts and acarbose at 125 μg/mL. **** $p < 0.0001$; *** $p = 0.0004$; ns = not significant.

2.3.2. IC50 Value of Teucrium Kotschyanum Essential Oil

Essential oil obtained via 3 h hydrodistillation from *T. kotschyanum* aerial parts (yield 0.11%, v/w) also tested for α-glucosidase inhibitory activity. A logarithmic curve was acquired (Figure 2), and the IC_{50} value was calculated according to the curve. *T. kotschyanum* EO (IC_{50} = 100.25 ± 8.38 μg/mL), possess significantly ($p<0.05$) higher activity than acarbose (122.03 ± 5.79 μg/mL).

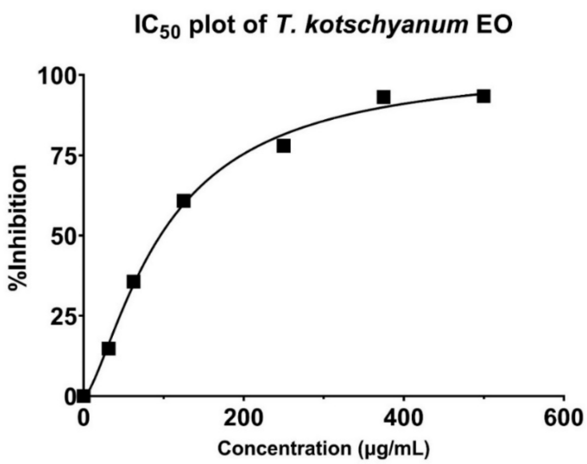

Figure 2. IC_{50} plot for α-glucosidase inhibition of T. kotschyanum EO.

3. Discussion

The essential oil from the aerial parts of *Teucrium kotschyanum* Poech was obtained by hydrodistillation, and the composition of this oil was analyzed by GC-MS. The yield of essential oils from the aerial parts of the plant was found to be 0.4%. The relative percentages and retention indices of the compounds in the essential oil are listed in Table 1.

53 constituents, representing 96.2% of the total components, were identified in the essential oil. The major compounds were identified as germacrene D (20.0%), α-cadinol (6.9%), β-bourbonene (6.6%), hexadecanoic acid (6.6%), δ-cadinene (5.9%), and manool (4.1%), other significant compounds were found as alloaromadendrene (3.0%), linalool (2.8%), T-muurolol (2.8%), viridiflorol (2.7%), T-cadinol (2.5%), and β-caryophyllene (2.0%).

Teucrium species are rich in essential oils, and the presence of various biologically active monoterpenoids and sesquiterpenoids with several biological activities has been reported. *Teucrium polium*, *T. chamaedrys*, *T. flavum* and *T. capitatum* are the most studied taxa in terms of volatile oil composition

 doi:10.20944/preprints202503.0806.v1

[31]. Phytochemical studies on the essential oils of *Teucrium* species showed that the major components of these oils are sesquiterpene hydrocarbons, such as germacrene D and β-caryophyllene, or monoterpene hydrocarbons, such as α- and β-pinene, and the taxa can be grouped into two different classes: sesquiterpene and monoterpene chemotypes [32].

Many *Teucrium* species growing in Türkiye have been investigated for their essential oil composition and the species *T. chamaedrys* [33], *T. chamaedrys* ssp. *chamaedrys* [34], *T. chamaedrys* ssp. *lydium* [34], *T. chamaedrys* ssp. *trapezunticum* [35], *T. cavernarum* [36], *T. paederotoides* [36], *T. antitauricum* [37], *T. montanum* [38], *T. polium* [39], *T. lamiifolium* ssp. *lamiifolium* [40], *T. multicaule* [41], *T. orientale* ssp. *orientale* [42], *T. orientale* ssp. *puberulens* [42]), *T. parviflorum* [43], *T. pestalozzae* [38] and *T. sandrasicum* [38] have been shown to contain germacrene D in their EOs as a major compound. The essential oils of *T. chamaedrys* subsp. *chamaedrys* and subsp. *lydium* growing northeast of Türkiye were found to contain germacrene D, α-pinene, β-caryophyllene, and β-pinene [34]. *T. cavernarum* and *T. paederotoides* which are endemic to Türkiye were reported to contain β-caryophyllene, germacrene D and caryophyllene oxide as major constituents in their essential oils [36]. In a previous study by Bezic et al. [44], it was reported that essential oils from Croation *Teucrium* species (*T. polium*, *T. flavum*, *T. montanum* and *T. chamaedrys*) were characterized by a high proportion of β-caryophyllene and germacrene D. The essential oil obtained from the flowering aerial parts of *T. scordium* collected from North Iran was found to contain β-caryophyllene as the major constituent [45]. The Corsican and Sardinian oils of *T. chamaedrys* were qualitatively similar, but differed according to the amount of their major components, β-caryophyllene and germacrene D [46]. The composition of the essential oil of *T. polium* subsp. *capitatum* from Corsica was investigated and the main components of the oil were found as α-pinene (28.8%), β-pinene (7.2%) and p-cymene (7.0%) [47]. In Greece, carvacrol (10.1%), caryophyllene (9.8%), torreyol (7.6%) and caryophyllene oxide (5.0%) were found to be the major constituents of *T. polium* ssp. capitatum essential oil [14].

The essential oils of *Teucrium* species were characterized by a higher content of sesquiterpenes, in accordance with the results reported in previous studies [30,34,48,49]. In this study, it has been shown that essential oil from the aerial parts of *T. kotschyanum* in İzmir-Türkiye contained germacrene D (20.0%), α-cadinol (6.9%), β-bourbonene (6.6%), hexadecanoic acid (6.6%), δ-cadinene (5.9%), and manool (4.1%) as the major constituents. Previously, the essential oil from the leaves of *T. kotschyanum* in Cyprus had a different profile, and its chemical composition was rich in β-cubebene, β-burbonene, and β-caryophyllene [23]. In this study, we have also detected β-burbonene (6.6%) in the essential oil of *T. kotschyanum*. Consequently, *T. kotschyanum* which is growing in İzmir- Türkiye could be classified among *Teucrium* species producing germacrene D as the main constituent such as other Turkish *Teucrium* species; *T. chamaedrys* [33], *T. chamaedrys* ssp. *chamaedrys* [34], *T. chamaedrys* ssp. *lydium* [34], *T. chamaedrys* ssp. *trapezunticum* [35], *T. cavernarum* [36], *T. paederotoides* [36], *T. antitauricum* [37], *T. montanum* [38], *T. polium* [39], *T. lamiifolium* ssp. *lamiifolium* [40], *T. multicaule* [41]; [50], *T. orientale* ssp. *orientale* [42], *T. orientale* ssp. *puberulens* [42], *T. parviflorum* [43], *T. pestalozzae* [38] and *T. sandrasicum* [38].

Extracts and essential oils from the *Teucrium* genus contain a wide range of secondary metabolites that exhibit biological activity. The antimicrobial activity of the essential oil obtained from the aerial parts of *Teucrium kotschyanum* from Türkiye (İzmir-Ödemiş) has not been previously reported. In this study, the antimicrobial activities of the essential oil were tested using the broth microdilution method on four strains of gram-positive bacteria (*S. aureus*, *S. epidermidis*, *B. cereus*, *B. subtilis*), two strains of gram-negative bacteria (*P. aeruginosa*, *S. thyphimurium*) and six strains of fungi (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis*, *C. utilis*). The essential oil showed moderate to weak inhibitory effects. The antimicrobial activities of the essential oils are shown in Table 2. MIC values of the essential oil against the selected human pathogenic bacteria and fungus were determined to be 125–2000 μg/mL.

The essential oil from the aerial parts of *T. kotshyanum* exhibited the highest antibacterial potency against *B. cereus* at 250 μg/mL. The least sensitive microorganisms to the essential oil from the aerial parts of *T. kotshyanum* were *S. epidermidis* and *S. thyphimurium* with 2000 μg/mL MIC values, respectively. The essential oil from the aerial parts of *T. kotshyanum* showed the strongest antifungal activity against *C. utilis* with an MIC value of 125 μg/mL.

In vitro antimicrobial activity of the crude extracts of *T. chamaedrys*, *T. montanum*, *T. arduini*, *T. polium*, *T. scordium* subsp. *scordium*, *T. scordium* subsp. *scordioides* and *T. botrys* from Serbia showed greater antibacterial potential than antifungal activity [51].

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 March 2025 doi:10.20944/preprints202503.0806.v1

The antimicrobial activities of the essential oils from *T. chamaedrys* subsp. *chamaedrys*, *T. orientale* var. *puberulens*, and *T. chamaedrys* subsp. *lydium* growing in Türkiye were tested in vitro using agar-well diffusion and the essential oils showed better antimicrobial activity against the gram-positive bacteria than against gram-negative bacteria [34].

The antimicrobial activity of *Teucrium* essential oils varies significantly within species and strains. *Teucrium africanum* essential oil showed noteworthy activity against *S. pyogenes* with an MIC value of 0.16 mg/mL while *T. trifidum* oil showed activity against *S. aureus* with an MIC value of 2 mg/mL [52]. The essential oil of *T. arduini* from Croatia which was characterized by β-caryophyllene (32.9%) and germacrene D (16.4%) showed antimicrobial activity against bacterial species with MIC values ranging from 6.25 mg/ml to 37.50 mg/mL and antifungal activities with MIC values from 7.81 mg/mL and 25.00 mg/mL [53]. The essential oil of aerial parts of *T. arduini* from Montenegro-Greben, which was characterized by sesquiterpene hydrocarbons (48.76%), germacrene D (16.98%), β-caryophyllene (14.98 %), β-burbonene (5.59%), and α-amorphene (4.68%), displayed a maximum activity against *K. pneumonia* with an MIC value of 6.25 μg/mL [54]. *T. divaricatum* which is a medicinal plant used in Lebanon, has been shown to contain abundant (E)- caryophyllene (30.1%) and caryophyllene oxide (6.1%) in its essential oil, the oil was found to have a good activity against gram-positive bacteria [55].

Teucrium montanum essential oil was analyzed and the occurrence of δ-cadinene (17.19%) and β-selinene (8.16%) were the major constituents was shown. According to the antimicrobial activity results, the highest inhibition zone was observed against *K. pneumoniae* (29 mm) [56]. The essential oil of *T. polium* from western Algeria was tested for antibacterial activity, and the highest activity was determined against *S. aureus* with an MIC value of 31.25 μL/mL [57]. The chemical composition of essential oil from Algerian *T. polium* was analyzed, and germacrene D (25.81%), bicyclogermacrene (13%), β-pinene (11.69%), and carvacrol (8.93%) were found as the major components, and antimicrobial activity test results of this species showed that the oil exhibited moderate inhibitory activity against *Bacillus cereus*, *Enterococcus faecalis*, *E. coli* and *S. aureus* with an MIC value of 3-5 μL/mL [58]. The essential oil of *T. polium* from Iran was analyzed, and β-caryophyllene (29%), farnesene (13%), β-pinene (11%), and germacrene D (6.5%) were detected as major components, in addition *T. polium* essential oil has shown to have a potential for use against multidrug resistant organisms such as clinical isolates of *K. pneumoniae* [59].

In a study from Morocco the essential oils of *T. polium* L. subsp. *polium* and *T. polium* L. subsp. *aureum* were tested against six bacteria strains responsible for nosocomial infections and oils from the species exhibited significant antimicrobial activity against *S. aureus* (MIC 0.17 mg/mL) and *K. pneumoniae* (MIC 1.4 and 0.7 mg/mL) but showed a low activity against Gram-negative *P. aeruginosa* [60]. The essential oil of *T. polium* L. from Tunisia was found to be rich in α- pinene (13.32%), β-pinene (35.97%), α-thujene (8.46%), p-cymene (5.25%), and verbenone (5.03%), and the essential oil exhibited high antimicrobial activity against *Proteus mirabilis*, *Staphylococcus aureus*, and *Citrobacter freundei* with MIC values of 0.078-0.156 mg/mL [61]. The main components of the essential oil of Algerian *T. polium* L. subsp. *geyrii* were identified as dl-limonene (11.18%), δ-cadinene (10.02%), and trans β-caryophyllene (9.15%), and the data obtained from the antimicrobial tests indicated that *C. albicans* was the most sensitive microorganism tested, followed by *S. aureus* and *E. coli* [62].

An endemic plant in Tunisia, *Teucrium sauvagei* was shown to have β-eudesmol, T-cadinol, α-thujone, γ-cadinene, and sabinene as the prevalent constituents in its essential oil, with the results indicating that the oil showed average inhibition against only three dermatophytes [63].

According to the literature, plants belonging *Teucrium* genus show antidiabetic activity according to in vivo and in vitro assays [64]. In one of these studies, different extracts of three *Teucrium* species were tested for α-amylase inhibition. The strongest activity was observed for the hydroalcoholic extracts of *T. polium* and *T. oliverianum*, with % inhibitions of 97.77% and 95.03%, respectively. The ethyl acetate extracts from *T. polium* and *T. oliverianum* have also high inhibitory activity with values of 83.41 %, 89.47% [65]. The blood glucose and serum insulin levels of the aqueous extract of *T. leucocladum* were investigated using a glucometer and Rat ELISA Kit. The extract reduces glucose levels in diabetic rats [66]. Sabet et al. [67] determined the antidiabetic activity of *T. polium* aqueous extracts in a streptozotocin-induced rat model of type 1 diabetes. *T. polium* extract caused a significant decrease in serum glucose levels. In another study, a new sesquiterpenoid compound isolated from *Teucrium mascatense* demonstrated significant inhibition in an α-glucosidase enzyme assay [68].

 doi:10.20944/preprints202503.0806.v1

In the current study, essential oil obtained from the aerial parts and extracts derived from the aerial and root parts of *T. kotschyanum* were investigated for the first time for their antidiabetic activity using the α-glucosidase inhibition method. EO exerts a valuable enzyme inhibitory potential. The findings showed that *T. kotschyanum* EO had a significant activity with an IC50 value of 100.25 μg/mL, compared to acarbose as a positive control (IC50 value of 122.03 μg/mL ($p<0.05$).

In previous studies, it has been determined that terpenic compounds have antidiabetic activity [69–71]. We suggest that enzyme inhibition activity is related to the presence of terpenic substances in the essential oil of this plant. Further studies are needed to define the exact mechanisms underlying the antidiabetic activities of the major compounds.

Additionally, our results revealed that RO-PE and RO-EA showed the best enzyme inhibitory effects with the inhibitions of 94.79% and 55.49%, respectively. AP-MeOH and RO-MeOH extracts exhibited potent effects with percent inhibition values (40.54% and 38.97%, respectively). Alamgeer et al. [72] evaluated the effect of ethyl acetate extract of *T. stocksianum* on blood glucose levels in alloxan-induced diabetic rabbits. Similar to our findings, the ethyl acetate extract showed the best antidiabetic effect. The extract produced a significant ($P<0.001$) decrease in the blood glucose levels. In another study, it was observed that, treatment of sucrose-induced insulin resistance in rats with ethyl acetate extract prepared from *T. polium* caused a significant decrease in blood glucose and insulin levels [73]. Results showed that the further studies are needed to identify the active compounds of both petroleum ether and ethyl acetate extracts that are responsible for this activity.

4. Materials and Methods

Plant Material

Teucrium kotschyanum Poech was collected from Ödemiş- İzmir in June 2014. The aerial parts of the plants were dried at room temperature. Voucher specimens were deposited at the Herbarium of the Faculty of the Pharmacy, Istanbul University, Istanbul (ISTE 109573).

Isolation of Essential Oil (EO)

The aerial parts (100 g) of *T. kotschyanum* Poech were chopped into small pieces and soaked in water (distilled, 500 mL). The essential oil from the air-dried plant materials was isolated by hydrodistillation for 3h, using a Clevenger-type apparatus. The obtained oil was dried over anhydrous sodium sulfate and stored at +4°C in the dark until analysis and testing.

Preparation of Extracts

Air-dried and powdered roots and aerial parts were exhaustively extracted using a Soxhlet apparatus with petroleum ether, ethyl acetate, and methanol. The resulting extracts were filtered and concentrated using a rotary evaporator under reduced pressure at 40°C. The filtered plant extracts were combined and stored at -20°C until the α-glucosidase inhibition assay.

Analysis of Essential Oil

The oils were analyzed by capillary Gas Chromatography (GC) and gas chromatography / mass chromatography (GC/MS) using an Agilent GC-MSD system.

Gas Chromatography/Mass Spectrometry Analysis

GC-MS analysis was performed using an Agilent 5975 GC-MSD system. Innowax FSC column (60 m × 0.25 mm, 0.25 mm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was maintained at 60°C for 10 min, programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min, and then increased to 240°C at a rate of 1°C/min. The split ratio was adjusted to 40:1. The injector temperature was set to 250°C. Mass spectra were recorded at 70 eV. The mass range was m/z 35 to 450.

Gas Chromatography Analysis

Gas chromatography (GC) analysis was performed using an Agilent 6890N GC system. The FID detector temperature was 300°C. To obtain the same elution order as GC-MS, simultaneous auto-injection was performed in duplicate on the same column under the same operational conditions. The

 doi:10.20944/preprints202503.0806.v1

relative percentage of the separated compounds were calculated from the FID chromatograms. Table 1 presents the results of the analysis.

The essential oil components were identified by comparing of their relative retention times with those of authentic samples or by comparison their relative retention index (RRI) to a series of n-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 4.0 Library) [74,75] and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils.

Antimicrobial Activity

The antimicrobial activity against Staphylococcus aureus ATCC 43300, Staphylococcus epidermidis ATCC 14990, Bacillus cereus NRRL B-3711, Bacillus subtilis NRRL B-4378, Pseudomonas aeruginosa ATCC 10145, Salmonella typhimurium ATCC 14028, Candida albicans ATCC 10231, Candida glabrata ATCC 2001, Candida krusei ATCC 6258, Candida parapsilosis ATCC 22019, Candida tropicalis ATCC 1369, and Candida utilis NRRL Y-900 was evaluated using partly modified CLSI microdilution broth methods M7-A7 and M27-A2, respectively (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria Grow Aerobically, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts) [76,77]. Chloramphenicol (Merck), ampicillin-Na (Merck), amphotericin-B (Sigma-Aldrich), and ketoconazole (Sigma-Aldrich) were used as the standard antimicrobial agents.

α-Glucosidase inhibition Activity

The α-glucosidase inhibitory activities of the samples were evaluated using the procedure described by Bothon et al. [78] with slight modifications. Samples (25 μL) were dissolved in DMSO and mixed with 50 μL α-glucosidase (1.0 U/mL) (from Saccharomyces cerevisiae) enzyme solution and 75 μL sodium phosphate buffer (0.1 M, pH 6.8) in a 96-well microplate. After incubation at 37°C for 10 min, 50 μL of substrate (p-nitrophenyl α-D-glucopyranoside) solution (1 mM) was added to the reaction mixture. Absorbance change was measured with Thermoscientific Multiscan® plate reader (Multiscan Go) at 405 nm wavelength and 37 °C for 10 min at 30 s intervals. Acarbose and DMSO were used as positive and negative controls respectively. % Inhibition values were calculated according to Eq. 1. The IC_{50} (concentration required to decrease the reaction rate to 50%) values were determined from the concentration-dependent curve.

Eq. 1: Inhibition (%) = [1 − (Absorbance sample/Absorbance control)] × 100

Statistical Analysis

The results are expressed as mean ± SEM values of at least three replicates for each sample. Inhibition (%) data of extracts were analyzed by one-way ANOVA followed by Dunnett-Tukey's multiple comparison tests, and IC50 values of essential oils were evaluated by unpaired t-test using GraphPad Prism (version 9.3.1 for Windows, GraphPad Software, San Diego, California, USA). Differences in group means ($p<0.05$) were considered significant.

5. Conclusions

The current study was undertaken to characterize and identify the chemical constituents of the essential oil from *T. kotschyanum* Poech and to evaluate its antimicrobial and antidiabetic activities. GC analysis of *T. kotshyanum* essential oil showed a chemical profile rich in germacrene D. The essential oil exhibited a moderate to weak antimicrobial activity. Alternatively, this species can be evaluated as a candidate for further examination to contribute to the treatment of diabetes mellitus.

Author Contributions: Conceptualization, participated in the investigation, writing, editing, Ç.Ü.G.; methodology, analysis, investigation, S.Y.T.; analysis, investigation, G.G.T.; methodology, analysis, investigation, G.İ.; methodology, analysis, investigation, B.D.; conceptualization, participated in the investigation, N.T. All authors have read and agreed to the published version of the manuscript

Funding: This research received no external funding

Data Availability Statement: Data supporting this study are included within the article and / or supporting materials.

Acknowledgments: The authors thank Hulusi Kütük for collecting the plant material.

 doi:10.20944/preprints202503.0806.v1

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

References

1. Guiche, R. E.; Tahrouch, S.; Amri, O.; El Mehrach, K.; Hatimie, A. Antioxidant Activity and Total Phenolic and Flavonoid Contents of 30 Medicinal and Aromatic Plants Located in the South of Morocco. *Int. J. New Technol. Res.* **2015**, *1* (7), 7–11.
2. Govaerts, R. *World Checklist Seed Plants 3*; Continental Publishing: Deurne, 1999.
3. Duman, H. *Teucrium L.* In *Flora of Turkey and the East Aegean Islands*; Güner, A., Özhatay, N., Ekim, T., Başer, K. H. C., Eds.; Edinburgh University Press: Edinburgh, 2000; Vol. 11, pp 197–198.
4. Dönmez, A. A. *Teucrium chasmophyticum* Rech. f. (Lamiaceae): A New Record for the Flora of Turkey. *Turk. J. Bot.* **2006**, *30*, 317–320.
5. Parolly, G.; Eren, Ö. Contributions to the Flora of Turkey, 2. *Wildenowia* **2007**, *37*(1), 243–271.
6. Dönmez, A. A.; Mutlu, B.; Özçelik, A. D. *Teucrium melissoides* Boiss. & Hausskn. ex Boiss. (Lamiaceae): A New Record for Flora of Turkey. *Hacettepe J. Biol. Chem.* **2010**, *38*, 291–294.
7. Dinç, M.; Doğu, S.; Bağcı, Y. Taxonomic Reinstatement of *Teucrium andrusi* from *T. paederotoides* Based on Morphological and Anatomical Evidences. *Nord. J. Bot.* **2011**, *29*, 148–158.
8. Dinç, M.; Doğu, S.; Doğru Koca, A.; Kaya, B. Anatomical and Nutlet Differentiation Between *Teucrium montanum* and *T. polium* from Turkey. *Biologia* **2011**, *66*, 448–453.
9. Dirmenci, T. *Teucrium L.* In *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*; Güner, A., Aslan, S., Ekim, T., Vural, M., Babaç, M. T., Eds.; Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırmaları Derneği Yayını: Istanbul, 2012; pp 595–598.
10. Özcan, T.; Dirmenci, T.; Coşkun, F.; Akçiçek, E.; Güner, A. A New Species of *Teucrium* sect. *Scordium* (Lamiaceae) from SE of Turkey. *Turk. J. Bot.* **2015**, *39*(2), 310–317.
11. Vural, M.; Duman, H.; Dirmenci, T.; Özcan, T. A New Species of *Teucrium* sect. *Stachyobotrys* (Lamiaceae) from the South of Turkey. *Turk. J. Bot.* **2015**, *39*(2), 318–324.
12. Vukovic, N.; Sukdolak, S.; Solujic, S.; Mihailovic, V.; Mladenovic, M.; Stojanovic, J.; Stankovic, M. S. Chemical Composition and Antimicrobial Activity of *Teucrium arduini* Essential Oil and Cirsimarin from Montenegro. *J. Med. Plant Res.* **2011**, *5*, 1244–1250.
13. Rehman, N. U.; Al-Sahai, J. M. S.; Hussain, H.; Khan, A. L.; Gilani, S. A.; Abbas, G.; Hussain, J.; Sabahi, J. N.; Al-Harrasi, A. Phytochemical and Pharmacological Investigation of *Teucrium muscatense*. *Int. J. Phytomed.* **2016**, *8*, 567–579.
14. Menichini, F.; Conforti, F.; Rigano, D.; Formisano, C.; Piozzi, F.; Senatore, F. Phytochemical Composition, Anti-inflammatory and Antitumour Activities of Four *Teucrium* Essential Oils from Greece. *Food Chem.* **2009**, *115*, 679–686.
15. Bellomaria, B.; Arnold, N.; Valentini, G. Essential Oil of *Teucrium flavum* subsp. *hellenicum* from Greece. *J. Essent. Oil Res.* **1998**, *10*, 131–133.
16. Ünsal, Ç.; Vural, H.; Sariyar, G.; Özbek, B.; Ötük, G. Traditional Medicine in Bilecik Province (Turkey) and Antimicrobial Activities of Selected Species. *Turk. J. Pharm. Sci.* **2010**, *7*(2), 139–150.
17. Sargın, S. A. Ethnobotanical Survey of Medicinal Plants in Bozyazı District of Mersin, Turkey. *J. Ethnopharmacol.* **2015**, *173*, 105–126.
18. Piozzi, F.; Riello, A.; Mazzini, F.; Senatore, F. Essential Oils of *Teucrium* Species from the Mediterranean Area. *Flavour Fragr. J.* **2003**, *18*, 136–141.
19. Ulubelen, A.; Topçu, G.; Sönmez, U. Chemical and Biological Evaluation of Genus *Teucrium*. *Stud. Nat. Prod. Chem.* **2000**, *23*, 591–648.
20. Ekim, T. *Teucrium L.* In *Flora of Turkey and the East Aegean Islands*; Davis, P. H., Ed.; Edinburgh University Press: Edinburgh, 1982; Vol. 7, pp 53–75.
21. Arnold, N. *Contribution à la Connaissance Ethnobotanique et Médicinale de la Flore de Chypre*. Université René Descartes: Paris, 1985; pp 1203–1210.
22. Simoes, F.; Rodríguez, B.; Bruno, M.; Piozzi, F.; Savona, G.; Arnold, N. A. Neo-clerodane Diterpenoids from *Teucrium kotschyanum*. *Phytochemistry* **1989**, *28*(10), 2763–2768.
23. Arnold, N.; Bellomaria, B.; Valentini, G.; Rafaiani, S. M. Comparative Study on Essential Oil of Some *Teucrium* Species from Cyprus. *J. Ethnopharmacol.* **1991**, *35*, 105–113.

 doi:10.20944/preprints202503.0806.v1

24. Jakovljević, D.; Stanković, M. Application of *Teucrium* Species: Current Challenges and Further Perspectives. In *Teucrium Species: Biology and Applications*; 2020; pp 413–432.
25. Esmaeili, M. A.; Yazdanparast, R. Hypoglycaemic Effect of *Teucrium polium*: Studies with Rat Pancreatic Islets. *J. Ethnopharmacol.* **2004**, *95*, 27–30.
26. Vahidi, A. R.; Dashti, R. M.; Bagheri, S. M. The Effect of *Teucrium polium* Boiled Extract in Diabetic Rats. *Iranian J. Diabetes Obesity* **2010**, *2*(2), 27–32.
27. Dastjerdi, Z. M.; Namjoyan, F.; Azemi, M. E. Alpha Amylase Inhibition Activity of Some Plant Extracts of *Teucrium* Species. *Eur. J. Biol. Sci.* **2015**, *7*, 26–31.
28. Asghari, A. A.; Mokhtari-Zaer, A.; Niazmand, S.; McEntee, K.; Mahmoudabady, M. Anti-diabetic Properties and Bioactive Compounds of *Teucrium polium* L. *Asian Pac. J. Trop. Biomed.* **2020**, *10*, 433.
29. Ali, N. A. A.; Wurster, M.; Arnold, N.; Lindequist, U.; Wessjohan, L. Chemical Composition of the Essential Oil of *Teucrium yemense* Deflers. *Records Nat. Prod.* **2008**, *2*(2), 25.
30. Saracoglu, V.; Arfan, M.; Shabir, A.; Hadjipavlou-Litina, D.; Skaltsa, H. Composition and Antioxidant Activity of the Essential Oil of *Teucrium royleanum* Wall. ex Benth Growing in Pakistan. *Flavour Fragr. J.* **2007**, *22*, 154–157.
31. De Martino, L.; Coppola, R.; De Feo, V.; Caputo, L.; Fratianni, F.; Nazzaro, F. Essential Oils Diversity of *Teucrium* Species. In *Teucrium Species: Biology and Applications*; Stanković, M., Ed.; Springer: Cham, 2020.
32. Candela, R. G.; Roselli, S.; Bruno, M.; Fontana, G. A Review of the Phytochemistry, Traditional Uses and Biological Activities of the Essential Oils of Genus *Teucrium*. *Planta Med.* **2020**, *87*, 432–479.
33. Bağcı, E.; Yazgın, A.; Hayta, S.; Çakılcıoğlu, U. Composition of the Essential Oil of *Teucrium chamaedrys* L. (Lamiaceae) from Turkey. *J. Med. Plants Res.* **2010**, *4*, 2588–2590.
34. Küçük, M.; Güleç, C.; Yaşar, A.; Üçüncü, O.; Yaylı, N.; Coşkunçelebi, K.; Yaylı, N. Chemical Composition and Antimicrobial Activities of the Essential Oils of *Teucrium chamaedrys* subsp. *chamaedrys*, *T. orientale* var. *puberulens*, and *T. chamaedrys* subsp. *lydium*. *Pharm. Biol.* **2006**, *44* (8), 592–599.
35. Kaya, A.; Demirci, B.; Başer, K. H. C. Compositions of Essential Oils and Trichomes of *Teucrium chamaedrys* L. subsp. *trapezunticum* Rech. fil. and subsp. *syspirense* (C. Koch) Rech. fil. *Chem. Biodivers.* **2009**, *6*, 96–104.
36. Kaya, A.; Demirci, B.; Dinç, M.; Doğu, S.; Başer, K. H. C. Compositions of the Essential Oils of *Teucrium cavernarum* and *Teucrium paederotoides*. *J. Essent. Oil-Bear. Plants* **2013**, *16* (5), 588–594.
37. Başer, K. H. C.; Demirci, B.; Duman, H.; Aytaç, Z. Composition of the Essential Oil of *Teucrium antitauricum* T. Ekim. *J. Essent. Oil Res.* **1999**, *11*, 61–62.
38. Başer, K. H. C.; Demirçakmak, B.; Duman, H. Composition of the Essential Oils of Three *Teucrium* Species from Turkey. *J. Essent. Oil Res.* **1997**, *9*, 545–549.
39. Hayta, S.; Yazgın, A.; Bağcı, E. Constituents of the Volatile Oils of Two *Teucrium* Species from Turkey. *Bitlis Eren Univ. J. Sci. Technol.* **2017**, *7*, 140–144.
40. Doğu, S.; Dinç, M.; Kaya, A.; Demirci, B. Taxonomic Status of the Subspecies of *Teucrium lamiifolium* in Turkey: Reevaluation Based on Macro- and Micro-Morphology, Anatomy and Chemistry. *Nord. J. Bot.* **2013**, *31*, 198–207.
41. Polat, T.; Özer, H.; Öztürk, E.; Çakır, A.; Kandemir, A.; Demir, Y. Chemical Composition of the Essential Oil of *Teucrium multicaule* Montbret et Aucher ex Bentham from Turkey. *J. Essent. Oil Res.* **2010**, *22* (5), 443–445.
42. 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.
43. Bağcı, E.; Hayta, S.; Yazgın, A.; Doğan, G. Composition of the Essential Oil of *Teucrium parviflorum* L. (Lamiaceae) from Turkey. *J. Med. Plant Res.* **2011**, *5* (15), 3457–3460.
44. Bezic, N.; Vuko, E.; Dunkic, V.; Ruscic, M.; Blazevic, I.; Burcul, F. Antiphytoviral Activity of Sesquiterpene-Rich Essential Oils from Four Croatian *Teucrium* Species. *Molecules* **2011**, *16*, 8119–8129.
45. Morteza-Semnani, K.; Saeedi, M.; Akbarzadeh, M. Essential Oil Composition of *Teucrium scordium* L. *Acta Pharm.* **2007**, *57* (4), 499–504.

 doi:10.20944/preprints202503.0806.v1

46. Muselli, A.; Desjobert, J. M.; Paolini, J.; Bernardini, A. F.; Costa, J.; Rosa, A.; Dessi, M. A. Chemical Composition of the Essential Oils of *Teucrium chamaedrys* L. from Corsica and Sardinia. *J. Essent. Oil Res.* **2009**, *21* (2), 138–143.
47. Cozzani, S.; Muselli, A.; Desjobert, J. M.; Bernardini, A. F.; Tomi, F.; Casanova, J. Chemical Composition of Essential Oil of *Teucrium polium* subsp. *capitatum* (L.) from Corsica. *Flavour Fragr. J.* **2005**, *20*, 436–441.
48. Cavaleiro, C.; Salgueiro, L. R.; Antunes, T.; Sevinate-Pinto, I.; Barroso, J. G. Composition of the Essential Oil and Micromorphology of Trichomes of *Teucrium salviastrum*, an Endemic Species from Portugal. *Flavour Fragr. J.* **2002**, *17*, 287–291.
49. Hachicha, S. F.; Skanji, T.; Barrek, S.; Zarrouk, H.; Ghrabi, Z. G. Chemical Composition of *Teucrium alopecurus* Essential Oil from Tunisia. *J. Essent. Oil Res.* **2007**, *19*, 413–415.
50. Ersoy, E.; Ozkan, E. E.; Karahan, S.; Şahin, H.; Cinar, E.; Canturk, Y. Y.; Boga, M. Phytochemical Analysis of Essential Oils and the Extracts of an Ethnomedicinal Plant, *Teucrium multicaule* Collected from Two Different Locations with Focus on Their Important Biological Activities. *S. Afr. J. Bot.* **2023**, *153*, 124–135.
51. Stanković, M. S.; Stefanović, O.; Čomić, L.; Topuzović, M.; Radojević, I.; Solujić, S. Antimicrobial Activity, Total Phenolic Content and Flavonoid Concentrations of *Teucrium* Species. *Cent. Eur. J. Biol.* **2012**, *7*, 664–671.
52. Ruiters, A. K.; Tilney, P. M.; Van Vuuren, S. F.; Viljoen, A. M.; Kamatou, G. P.; Van Wyk, B. E. The Anatomy, Ethnobotany, Antimicrobial Activity and Essential Oil Composition of Southern African Species of *Teucrium* (Lamiaceae). *S. Afr. J. Bot.* **2016**, *102*, 175–185.
53. Kremer, D.; Müller, I.; Dunkić, V.; Vitali, D.; Stabentheiner, E.; Oberländer, A.; Kosalec, I. Chemical Traits and Antimicrobial Activity of Endemic *Teucrium arduini* L. from Mt Biokovo (Croatia). *Open Life Sci.* **2012**, *7* (5), 941–947.
54. Vukovic, N.; Sukdolak, S.; Solujić, S.; Mihailovic, V.; Mladenović, M.; Stojanovic, J.; Stankovic, M. Chemical Composition and Antimicrobial Activity of *Teucrium arduini* Essential Oil and Cirsimarin from Montenegro. *J. Med. Plants Res.* **2011**.
55. Formisano, C.; Napolitano, F.; Rigano, D.; Arnold, N. A.; Piozzi, F.; Senatore, F. Essential Oil Composition of *Teucrium divaricatum* Sieb. ssp. *villosum* (Celak.) Rech. fil. Growing Wild in Lebanon. *J. Med. Food.* **2010**, *13* (5), 1281–1285.
56. Vukovic, N.; Milosevic, T.; Sukdolak, S.;Solujic, S. Antimicrobial Activities of Essential Oil and Methanol Extract of *Teucrium montanum*. *Evid-Based Complementary Altern Med*. **2007**, *4*, 368170-368173.
57. Fertout-Mouri, N.; Latrèche, A.; Mehdadi, Z.; Toumi-Benali, F.; Khaled, M. B. Chemical Composition and Antibacterial Activity of the Essential Oil of *Teucrium polium* of Tessala Mount (Western Algeria). *Phytothérapie* **2017**, *15*, 346–353.
58. Belmekki, N.; Bendimerad, N.; Bekhechi, C.; Fernandez, X. Chemical Analysis and Antimicrobial Activity of *Teucrium polium* L. Essential Oil from Western Algeria. *J. Med. Plants Res.* **2013**, *7* (14), 897–902.
59. 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* (1), 65–69.
60. 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* (2), 3866–3874.
61. Ben Othman, M.; Bel Hadj Salah-Fatnassi, K.; Ncibi, S.; Elaissi, A.; Zourgui, L. Antimicrobial Activity of Essential Oil and Aqueous and Ethanol Extracts of *Teucrium polium* L. subsp. *gabesianum* (L.H.) from Tunisia. *Physiol. Mol. Biol. Plants* **2017**, *23*, 723–729.
62. Roukia, H.; Mahfoud, H. M.; Didi, O. E. H. M. Chemical Composition and Antioxidant and Antimicrobial Activities of the Essential Oil from *Teucrium polium geyrii* (Labiatae). *J. Med. Plants Res.* **2013**, *7* (20), 1506–1510.
63. Bel Hadj Salah, K.; Mahjoub, M. A.; Chaumont, J. P.; Michel, L.; Millet-Clerc, J.; Chraeif, I.; Aouni, M. Chemical Composition and in vitro Antifungal and Antioxidant Activity of the Essential Oil and Methanolic Extract of *Teucrium sauvagei* Le Houerou. *Nat. Prod. Res.* **2006**, *20* (12), 1089–1097.

64. Asghari, A. A.; Mokhtari-Zaer, A.; Niazmand, S.; McEntee, K.; Mahmoudabady, M. Anti-Diabetic Properties and Bioactive Compounds of *Teucrium polium* L. *Asian Pac. J. Trop. Biomed.* **2020**, *10*, 433.
65. Dastjerdi, Z. M.; Namjoyan, F.; Azemi, M. E. Alpha Amylase Inhibition Activity of Some Plant Extracts of *Teucrium* Species. *Eur. J. Biol. Sci.* **2015**, *7*, 26–31.
66. Bassalat, N.; Tas, S.; Jaradat, N. *Teucrium leucocladum*: An Effective Tool for the Treatment of Hyperglycemia, Hyperlipidemia, and Oxidative Stress in Streptozotocin-Induced Diabetic Rats. *Evid.-Based Complement. Altern. Med.* **2020**, *1*, 1–8.
67. Sabet, Z.; Roghani, M.; Najafi, M.; Maghsoudi, Z. Antidiabetic Effect of *Teucrium polium* Aqueous Extract in Multiple Low-Dose Streptozotocin-Induced Model of Type 1 Diabetes in Rat. *J. Basic Clin. Pathophysiol.* **2013**, *1*, 32–36.
68. Rizvi, T. S.; Hussain, I.; Ali, L.; Mabood, F.; Khan, A. L.; Shujah, S.; Halim, S. A. New Gorgonane Sesquiterpenoid from *Teucrium mascatense* Boiss, as α-Glucosidase Inhibitor. *S. Afr. J. Bot.* **2019**, *124*, 218–222.
69. Majouli, K.; Hlila, M. B.; Hamdi, A.; Flamini, G.; Jannet, H. B.; Kenani, A. Antioxidant Activity and α-Glucosidase Inhibition by Essential Oils from *Hertia cheirifolia* (L.). *Ind. Crops Prod.* **2016**, *82*, 23–28.
70. Aba, P. E.; Asuzu, I. U. Mechanisms of Actions of Some Bioactive Anti-Diabetic Principles from Phytochemicals of Medicinal Plants: A Review. *Indian J. Nat. Prod. Resour.* **2018**, *9*, 85–96.
71. Adefegha, S. A.; Olasehinde, T. A.; Oboh, G. Essential Oil Composition, Antioxidant, Antidiabetic and Antihypertensive Properties of Two *Afromomum* Species. *J. Oleo Sci.* **2017**, *66*, 51–63.
72. Alamgeer, M.; Rashid, M. N.; Mushtaq, M. N. H.; Malik, S. A.; Ghumman, M.; Numan, A. Q.; Khan, M. O.; Omer; Tahir, H. M**.** Pharmacological Evaluation of Antidiabetic Effect of Ethyl Acetate Extract of *Teucrium stocksianum* Boiss in Alloxan-Induced Diabetic Rabbits. *J. Anim. Plant Sci.* **2013**, *23*, 436–439.
73. Mousavi, S. E.; Shahriari, A.; Ahangarpour, A.; Vatanpour, H.; Jolodar, A. Effects of *Teucrium polium* Ethyl Acetate Extract on Serum, Liver and Muscle Triglyceride Content of Sucrose-Induced Insulin Resistance in Rat. *Iran. J. Pharm. Res.* **2012**, *11* (1), 347.
74. McLafferty, F. W.; Stauffer, D. B.; Stenhagen, E.; Heller, S. R. *The Wiley/NBS Registry of Mass Spectral Data*; **1989.**
75. Hochmuth, D. H. *MassFinder 4.0*; Hochmuth Scientific Consulting, Hamburg, Germany, **2008**.
76. CLSI (NCCLS) M7-A7**.** Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, Seventh Edition, **2006**.
77. CLSI (NCCLS) M27-A2. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard, Second Edition, **2002**.
78. Bothon, F. T. D.; Debiton, E.; Avlessi, F.; Forestier, C.; Teulade, J. C.; Sohounhloue, D. K. In Vitro Biological Effects of Two Anti-Diabetic Medicinal Plants Used in Benin as Folk Medicine. *BMC Complement. Altern. Med.* **2013**, *13*, 51.