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RESEARCH ARTICLE

Phytochemical, Taxonomical and Biological Activity Studies on The Endemic *Salvia tchihatcheffii* Boiss.

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SUMMARY

The Lamiaceae family has many taxonomically and economically important plant species. The species in this family exhibit antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory properties. Due to these effects, it is used in the food and pharmaceutical industry. Salvia tchihatcheffii Boiss, an endemic species belonging to the Lamiaceae family, was collected from Eskişehir. In morphological studies, herbarium specimens were examined under a stereo microscope. Stem and leaf were examined within the scope of anatomical studies. Molecular studies aimed to determine the phylogenetic relationships of the plant. The antioxidant properties were determined by the DPPH• free radical scavenging and ABTS•+test, and the total phenolic content was calculated using the Folin-Ciocalteu method. Antimicrobial activity was evaluated using the Broth dilution method. Essential oil was obtained from the flowering aerial parts of the plant by hydrodistillation and was analyzed using GC-GC/MS. The morphological and anatomical findings obtained are compared with the characteristics listed in The Flora of Turkey. According to molecular analysis results, S. tchihatcheffii and S. cedronella were determined to be closely related. As a result of essential oil analysis, the main component was identified as camphor with 23.3%. The antimicrobial activities of extracts prepared from the aerial parts with n-hexane, chloroform, and ethanol were found to be at low levels compared to standard reference drugs. No antibacterial activity was observed at any concentration of the extracts against Escherichia coli and Pseudomonas aeruginosa strains. The n-hexane extract exhibited the highest total phenol content and DPPH• radical scavenging activity.

Keywords: *Salvia tchihatcheffii, morphology, anatomy, biological activity, ITS (Internal Transcribed Spacer), essential oil.*

Endemik Salvia tchihatcheffii Boiss. Üzerinde Fitokimyasal, Taksonomik ve Biyolojik Aktivite Çalışmaları

ÖZ

Lamiaceae familyası, taksonomik ve ekonomik açıdan önemli birçok bitki türü içermektedir. Bu familyadaki türler antibakteriyel, antifungal, antiviral, antioksidan ve antiinflamatuar özellikler göstermektedir. Bu etkileri nedeniyle gıda ve ilaç endüstrisinde kullanılmaktadır. Lamiaceae familyasına ait endemik bir tür olan Salvia tchihatcheffii Boiss, Eskişehir'den toplanmıştır. Morfolojik çalışmalarda, herbaryum örnekleri stereo mikroskop altında incelenmiştir. Anatomik çalışmalar kapsamında gövde ve yaprak incelenmiştir. Moleküler çalışmalar, bitkinin filogenetik ilişkilerini belirlemeyi amaçlamıştır. Antioksidan özellikleri DPPH• serbest radikal süpürme ve ABTS•+ testi ile belirlenmiş, toplam fenolik madde içeriği ise Folin-Ciocalteu yöntemiyle hesaplanmıştır. Antimikrobiyal aktivite, Broth mikrodilüsyon yöntemi kullanılarak değerlendirilmiştir. Bitkinin çiçekli toprak üstü kısımlarından hidrodistilasyon yöntemiyle uçucu yağ elde edilmiş ve GC-GC/MS yöntemiyle analiz edilmiştir. Elde edilen morfolojik ve anatomik bulgular, Türkiye Florası'nda listelenen özelliklerle karşılaştırılmıştır. Moleküler analiz sonuçlarına göre, S. tchihatcheffii ve S. cedronella'nın yakından ilişkili olduğu belirlenmiştir. Uçucu yağ analizi sonucunda, ana bileşenin %23,3 oranında kafur olduğu tespit edilmiştir. Bitkinin toprak üstü kısımlarından n-hekzan, kloroform ve etanol ile hazırlanan ekstrelerin antimikrobiyal aktivitesinin, standart referans ilaçlara kıyasla düşük seviyelerde olduğu bulunmuştur. Ekstrelerin hiçbir konsantrasyonunda Escherichia coli ve Pseudomonas aeruginosa suşlarına karşı antibakteriyel aktivite gözlenmemiştir. Hekzan ekstresi en yüksek toplam fenol içeriğine ve DPPH• radikal temizleme aktivitesi göstermiştir.

Anahtar Kelimeler: *Salvia tchihatcheffii, morfoloji, anatomi, biyolojik aktivite, ITS (Internal Transcribed Spacer), uçucu yağ.*

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INTRODUCTION

Plants have been used by humans for various purposes since the existence of the world. From ancient times, plants in nature have been utilized primarily for food, as well as for spices, medicines, and cosmetics (Acıbuca & Budak, 2018).

Türkiye is known for its rich biodiversity. The reasons for Türkiye's rich flora include its position at the intersection of three phytogeographical regions known as the Euro-Siberian, Mediterranean, and Irano-Turanian, making Anatolia a bridge between the flora of South Europe and Southwest Asia. In addition to its geographical location, the occurrence of different climatic conditions and geological structures also contributes to its importance as a floristic region (Davis, 1965; Davis & Hedge, 1975).

The flora of Türkiye is represented by over 11000 taxa belonging to 173 families and 1225 genera. New species continue to be discovered and added to the literature. Türkiye is also rich in endemic plant species, with 3500 out of the 11000 taxa being endemic (Davis, 1965; Towsend, 1973; Güner et al., 2012; Selvi et al., 2016).

Salvia L., the most important and widely distributed genus of the *Lamiaceae* family, has been used around the world for centuries. There are more than 900 species of the genus *Salvia* distributed in Central Asia, Africa, America, and the Mediterranean regions (Hedge, 1992; Bonesi et al., 2017). Anatolia and Asia are important centers of diversity for *Salvia,* and there are about 95 *Salvia* species in Türkiye, of which 49 (52%) are endemic (Towsend, 1973; Kahraman et al., 2010). *Salvia* species are widely used by the public and are also used in perfumery, cosmetics, and medicine (Nash, 2001). Research shows that *Salvia* species have therapeutic properties for digestive and circulatory disorders, asthma, bronchitis, cough, mouth and throat inflammations, excessive sweating, depression, dementia, and obesity (Hamidpour et al., 2014; Bonesi et al., 2017). *Salvia* species exhibit antioxidant, antidiabetic, antibacterial, antitumor, antispasmodic, and anti-inflammatory properties (Ulubelen, 2003; Kamatou et al., 2008; Kahraman et al., 2010). These species are important due to their high content of chemical compounds such as flavonoids, terpenoids, and essential oils, but especially the high content of essential oils makes this genus of great importance. In addition, they have been used since ancient times as antiseptics, antibacterials, sedatives, memory enhancers, and for certain menopause problems (Baytop, 1999; Nash, 2001; Hamidpour et al., 2014).

In this study, it was aimed to determine the morphological, anatomical characteristics, antioxidant and antibacterial activities, molecular phylogenetic relationships, and essential oil content of the endemic *Salvia tchihatcheffii* Boiss. from the floristically rich Eskişehir region.

MATERIALS AND METHODS

Plant material and extraction

Salvia tchihatcheffii collected from Kızılkayalar, Eskişehir on July 2, 2019 at 39°40'38" N 30°23'59" coordinates. The plant specimens are kept in the Herbarium of the Faculty of Pharmacy at Anadolu University (ESSE 15446). The plant was identified by Prof. Dr. Yavuz Bülent Köse.

The dry aerial parts of the plant were cut into small pieces and weighed 20 grams each. *n*-Hexane, ethyl alcohol, and chloroform solvents were added at a ratio of 1:10, and the samples were macerated at 150 rpm for 48 hours. The resulting macerates were filtered with the help of filter paper. This process was repeated three times. The extracts were evaporated at 40 °C under low pressure using a rotary evaporator. The extracts were stored in a refrigerator at +4 °C until use.

Morphological studies

Twenty herbarium specimens were prepared to identify plants and determine morphological char-

acteristics. Plant parts were examined under a stereo microscope (Nikon SMZ645). Morphological terminology was based on Prof. Dr. Asuman Baytop's botanical guide and compared with information from the "Flora of Turkey and the East Aegean Islands" (Davis, 1965; Baytop, 1998).

Anatomical studies

During field studies, all plant specimens were collected in glass jars containing 70% ethyl alcohol for anatomical examinations. Transverse and superficial sections were taken from the stem and leaf of the plant samples using a razor blade and clarified with chloral hydrate. The sections were stained with Sartur's reagent, phloroglucinol, and 25% HCl. The prepared sections were then photographed under a trinocular microscope (Olympus BX51T).

Molecular studies

Genomic DNA was isolated from plant samples using the EurX GeneMATRIX Plant & Fungi DNA Isolation Kit (Poland), and DNA concentration and purity were assessed with a Thermo Scientific Nanodrop 2000 spectrophotometer (USA). Target regions were amplified by PCR for species identification. The primer sequences used and PCR conditions are given in Tables 1 and 2 (Innis et al. 1990).

Table 1. Forward (F) and reverse (R) primer sequences

ITS1	TCCGTAGGTGAACCTGCGG (F)
ITS2	TCCTCCGCTTATTGATATGC (R)
ITS3	GCTGCGTTCTTCATCGATGC (F)
ITS4	GGAAGTAAAAGTCGTAACAAGG (R)

ITS: Internal Transcribed Spacer

Table 2. PCR conditions

- 95 °C 5 minutes- initial denaturation
- 40 cycles
  - 45 seconds at 95 °C - denaturation
  - 45 seconds 57 °C - annealing
  - 60 seconds 72 °C - extension
- 72 °C 5 minutes - final extension
- The temperature was reduced to 4 °C and PCR was completed.

The amplification results obtained by PCR (kyratec thermocycler) were electrophoresed in a 1.5% agarose gel. A one-step PCR was performed to amplify the region of approximately 700 and 350 bases. For our samples, a single band was obtained on the agarose gel after PCR. At the PCR product purification stage, the single band samples were purified using the MAGBIO "High Prep™ PCR Clean-up System" (AC-60005) purification kit, following the kit's procedures. For Sanger Sequencing samples, an ABI3730XL Sanger sequencer (Applied Biosystems, Foster City, CA) and BigDyeTerminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA.) were used at Macrogen Netherlands laboratory. The reads obtained with primers ITS1-ITS4 were contig-spliced to generate a consensus sequence. The CAP contig assembly algorithm in BioEdit software was used for this process. Sequence analyses were carried out by the BM Labosis company, and close relatives of the species were found with the Blast program by using the sequences.

Evolutionary analysis by the Maximum Likelihood method

The phylogeny was inferred using the Maximum Likelihood method and the Tamura and Nei (1993) model of nucleotide substitutions, and the tree with the highest log likelihood is shown (Tamura & Nei, 1993).

The percentage of replicate trees in which the associated taxa clustered together (1,000 replicates) is shown next to the branches (Felsenstein, 1985). The analytical procedure encompassed 43 nucleotide sequences. The partial deletion option was applied to eliminate all positions with less than 95% site coverage, resulting in a final dataset comprising 588 positions. Evolutionary analyses were conducted in MEGA12 utilizing up to 8 parallel computing threads (Kumar et al., 2024).

Essential oil studies and GC-GC/MS analysis

The essential oil was obtained from 20 g of herb by the water distillation method in the Clevenger apparatus. Essential oil accumulated in the collection container was removed with *n*-hexane. The essential oil was analyzed by GC-GC/MS (Gas Chromatography-Gas Chromatography/Mass Spectrometry). The analytical parameters for GC-GC/MS were adopted from the previously reported protocol in the relevant study (Agiel et al., 2024). Identification of the essential oil components was carried out by comparing their relative retention times with authentic samples or by comparing their relative retention indices (RRI) with *n*-alkane series. The evaluation was performed using the Wiley GC/MS Library, MassFinder Software 4.0, and the in-house "Başer Essential Oil Constituents Library"(Mclafferty & Stauffer, 1989; Hochmuth, 2008).

Determination of total phenolic content

The total phenolic content of extracts was calculated as gallic acid equivalent by the Folin-Ciocalteu method (Singleton et al., 1999; Agiel et al., 2024). Measurements were conducted at a wavelength of 760 nm. The analysis was performed in triplicate. The total phenolic content was determined using a gallic acid standard curve.

Determination of scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH●)

The DPPH radical scavenging effect was determined using a modified version of a previously established protocol (Kumarasamy et al., 2007). Statistical evaluations were performed using the Gen5 Data Analysis program (BioTek), and results were expressed as mean ± standard deviation. For radical scavenging activity, 50% inhibition concentration value (IC_{50}) was calculated, and the following formula was used for the % inhibition value. IC_{50} values were determined using nonlinear regression curves (Sigma Plot version 13, SPSS Inc., Chicago, IL) and are given as the average of three parallel experiments.

$$IC_{50} = [(A_0 - A_1) / A_0)] \times 100$$

IC_{50}: %50 Inhibitor concentration, A_0: Control absorbance, A_1: Sample absorbance

2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation ($ABTS^{\bullet+}$) scavenging effect

The experiment was conducted using the standard Trolox equivalent antioxidant capacity (TEAC) method. This method is based on the scavenging of $ABTS^{\bullet+}$ (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cation) and comparison with Trolox, a water-soluble analog of vitamin E (Papandreou et al., 2006).

Antimicrobial studies

Antimicrobial activity was tested against *Escherichia coli* NRRL B-3008, *Staphylococcus aureus* subsp. *aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Serratia marcescens* NRRL B-2544, and *Klebsiella pneumoniae* NCTC 9633. Antifungal activity was determined against *Candida albicans* ATCC 10231, *C. utilis* NRRL Y-900, *C. albicans* ATCC 90028, *C. tropicalis* ATCC 750, *C. parapsilosis* ATCC 22019, and *C. krusei* ATCC 6258 strains. In this study, antibacterial activity tests were performed using the partially modified CLSI

(NCCLS) M7-A7 protocol in accordance with clinical laboratory standards (CLSI M7-A7, 2006). The anticandidal effect of the extracts was assessed using the CLSI M27-A2 Broth microdilution protocol (CLSI M27-A2, 2002). Standards and extracts were dissolved in sterile DMSO. The minimum inhibitory concentration (MIC) values of the extracts were determined by the microdilution method. Amphotericin B, Ketoconazole, Ampicillin, and Chloramphenicol were used as standard antifungal and antibacterial agents.

RESULTS AND DISCUSSION

Morphology characteristics

The semi-shrub-like plant is a perennial. Sterile stems decumbent, leafy; fertile stems ascending, unbranched, 18-40 cm, with simple short hairs and sessile glandular hairs (Figure 1.). Leaves with 3 segments deep to mid-vein of lamina or 2 pairs of lateral segments with pinnate vein with lobes deep to mid-vein of leaf blade, terminal segment oblong with rounded corners, 1-2.5 × 0.2-0.6 cm, more or less naked with dense sessile glandular hairs, entire or terminal segment toothed at apex; petiole 3-8 mm, ciliate. Inflorescence spica and inflorescence rings 2-8 flowered, flowers 3-4 mm, pedicellate. Bracts ovate-acuminate, 10-16 × 4-7 mm, bracteoles present. Pedicels small, 3-4 mm. Calyx tubular, campanulate, 8-11 mm. Fruit 9-12 mm, sessile, glandular, and simply hairy-ciliate. Corolla light violet to white, 10-15 mm with tube 7-10 mm, ringed. Upper lip nearly straight. Nutlets rounded, triangular, ovate, 3 × 2.3 mm.

The morphological findings obtained for *S. tchihatcheffii* are compared with the characteristics listed in "The Flora of Turkey and the East Aegean Islands" in Table 3. The findings of the description study are very similar to the morphological characteristics and measurements in the Flora of Turkey. This study, along with a previously conducted morphological study, contributed to the literature. The findings obtained in this study were consistent with existing data (Aktas et al., 2009).

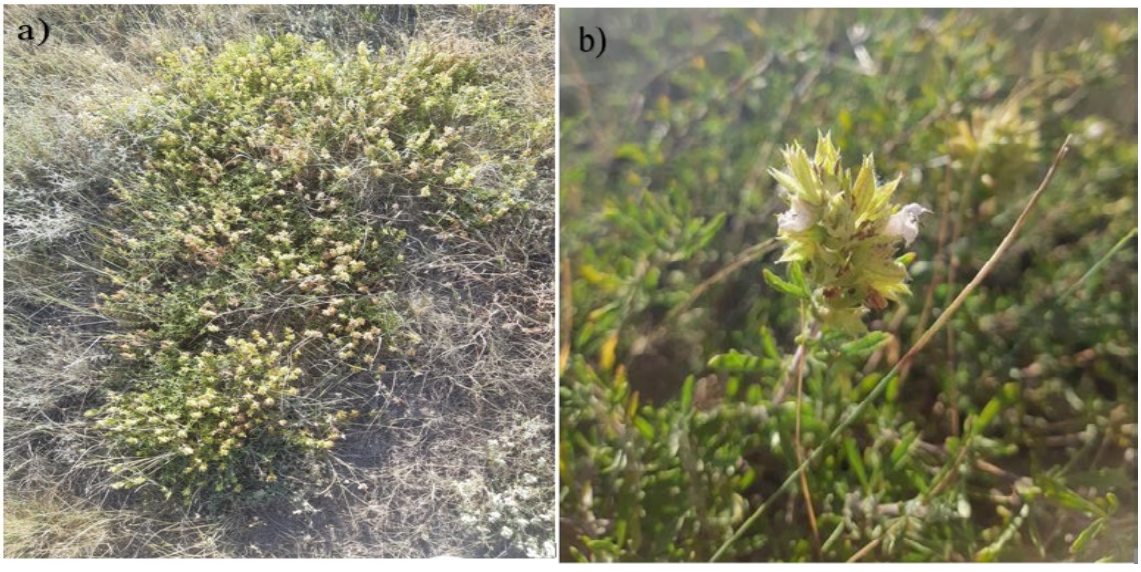

Figure 1. a) General appearance of *S. tchihatcheffii*, b) Inflorescence of *S. tchihatcheffii*

Table 3. Comparison of morphological characteristics of *S. tchihatcheffii*

Characters	Findings	Flora of Turkey (Vol. 7)
Plant height	18-40 cm	10-20 cm
Leaf	0.7-3 × 0.2-0.5 cm	1-2.5 × 0.2-0.6 cm
Petiole	0.3-0.4 cm	0.3-0.4 cm
Flower	Spika	Spika
Corolla colour	Very light violet, white	White with light violet
Calyx	1-1.5 × 1.2-1.5 cm	0.9-1.2 cm
Corolla	1-2 cm	1-1.5 cm
Life form	Semi-shrub, herbaceous	Semi-shrub, herbaceous
Hair	Simple and glandular hair	Simple and glandular hair

Anatomical studies

Stem anatomy

The transverse section taken from the stem of *S. tchihatcheffii* is squarish-round. The outermost layer is the epidermis, which consists of 1-2 rows of flat oval cells. There is a thick cuticle layer above the epidermis. The hairs on the epidermis are glandular. The cortex consists of three distinct layers. The first layer is composed of thick-walled, flattened collenchyma cells. The cells in the second layer are oval, crescent-shaped, and in 3-5 rows. The cells in the third layer consist of rows of cells of almost the same length and width, with no spaces between them. *S. tchihatcheffii* stem section is shown in Figure 2.

Leaf anatomy

The leaf has a single layer of epidermis with flat-ovoidal cells on the axis-facing and off-axis surface of the leaf. On the axis-facing surface of the epidermis, the cells are larger and more numerous. It has both 2-4 rows of palisate parenchyma cells and spongy cells, and has 3-5 layers. Hairs are present in the axial, adaxial, and extra-axial epidermis. There are both glandular hairs and glandless hairs. The leaf cross-section of *S. tchihatcheffii* is shown in Figure 3. Anatomical sections of *S. tchihatcheffii* show glandular hairs on the leaf, petiole, and calyx. Glandular hairs are capitate hairs. The capitate hair structure varies in size and proportion.

When the data from the anatomy study was compared with the data from Aktas et al. (2009) it was seen that the results were similar. It was stated that there were 2 types of stems and that they differed anatomically from each other. In the example we examined, the anatomical features of the sterile stem were defined. In the hair anatomy, it was seen that there were glandular hairs in various parts of the plant and that the features of these hairs were different (Aktas et al., 2009).

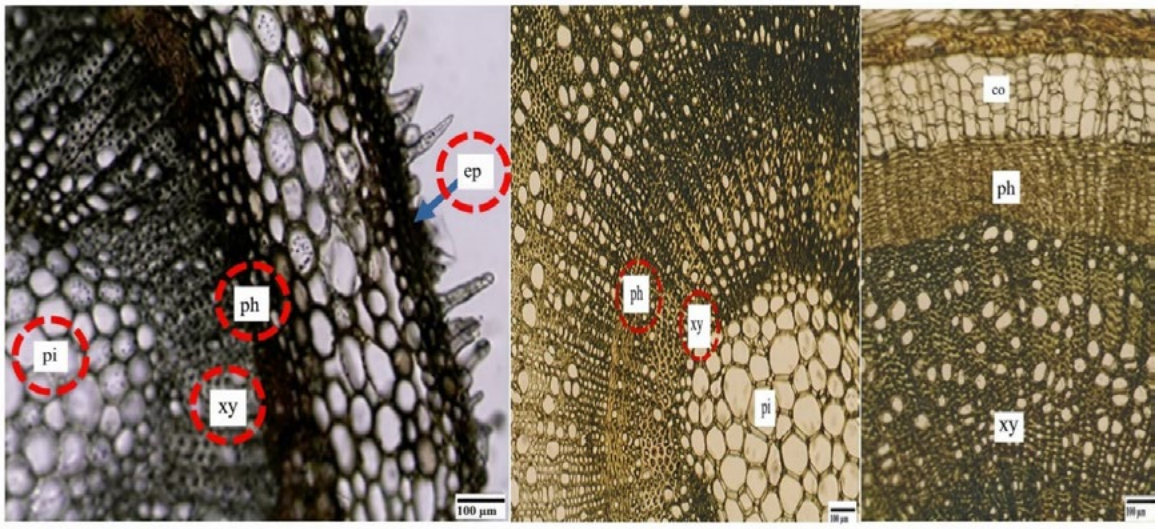

Figure 2. General view of the stem cross-section of *S. tchihatcheffii*; ep: epidermis, ph: phloem, xy: xylem; pi: pith, co: cortex

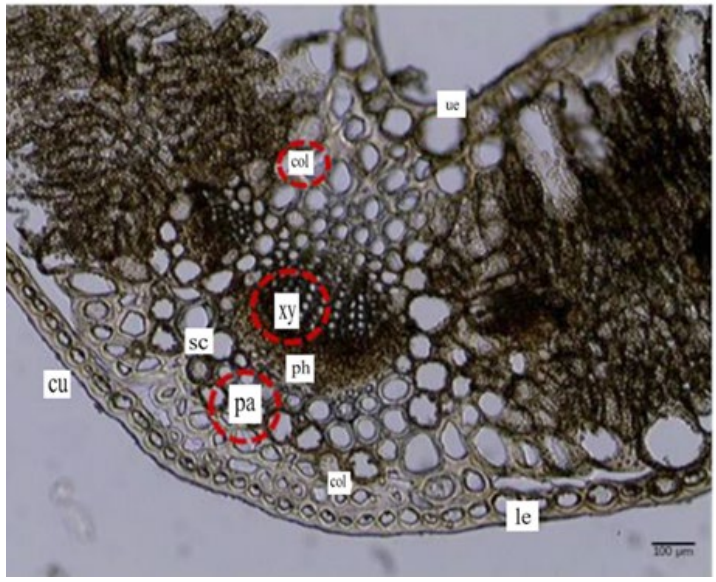

Figure 3. Leaf cross-section of *S. tchihatcheffii*: xy: xylem, ph: phloem, pa: parenchyma, col: collenchyma, sc: sclerenchyma, cu: cuticle, ue: upper epidermis, le: lower epidermis,

Molecular study

According to the results of DNA sequence analysis, the ITS sequences of *S. tchihatcheffii* were determined and compared with those of *Salvia* species in GenBank. *S. tchihatcheffii* was found to have a similarity rate of more than 94% with species belonging to the genus *Salvia* in GenBank (Table 4-5.). Building on these sequence comparisons, the kinship relationships of S. *tchihatcheffii* were revealed in this study. It was observed that *S. tchihatcheffii* and *S. cedronella* are closely related (Figure 4.).

Table 4. The Closest representatives of sequences obtained from of *Salvia* genus in GenBank and similarity rates

Similar Species in the Gene Bank	Similarity Rate %	Sequence Affinity Ratio	GenBank Number
S. officinalis	97.16%	650/669(97%)	ON685398.1
S. cedronella	99.67%	608/610(99%)	KU563797.1
S. tchihatcheffii	99.67%	609/611(99%)	KU563814.1
S. multicaulis	96.86%	648/669 (%97)	KP294353.1
S. pisidica	99.03%	610/616(99%)	MW221072.1
S. potentillifolia	99.03%	610/616(99%)	MW221062.1
S. albimaculata	99.03%	610/616(99%)	MW221073.1
S. adenophylla	99.18%	605/610(99%)	KU563789.1
S. nydeggeri	99.02%	604/610(99%)	KU563803.1
S. hydrangea	96.12%	644/670 (%96)	KP294355.1
S. heldreichiana	98.85%	603/610(99%)	KU563799.1
S. korolkovii	96.50%	634/657 (%96)	MW588812.1
S. cadmica	98.20%	599/610 (%98)	KU563795.1
S. candelabrum	97.70%	596/610(98%)	KJ584190.1
S. smyrnaea	97.70%	596/610(98%)	KU563812.1
S. ringens	97.24%	599/616(97%)	MW221064.1
S. interrupta	97.38%	594/610(97%)	KJ584191.1
S. submutica	95.15%	628/660(95%)	MW588814.1
S. fruticosa	95.28%	626/657(95%)	MW192836.1
S. pseudeuphratica	97.05%	593/611 (%97)	KM519762.1
S. pomifera	95.78%	613/640 (%96)	MW192850.1
S. rugosa	94.82%	623/657 (%95)	DQ667290.1
S. scabiosifolia	96.73%	592/612 (%97)	KU563811.1
S. euphratica	96.73%	591/611 (%97)	KU563819.1
S. tomentosa	96.58%	593/614 (%97)	KU563816.1
S. cerinopruinosa	96.56%	590/611 (%97)	KM519758.1
S. kurdica	96.56%	589/610 (%97)	KU563821.1
S. aramiensis	96.42%	592/614 (%96)	KU563791.1
S. kronenburgii	96.40%	589/611 (%96)	KM519759.1
S. dolomitica	94.50%	618/654 (%94)	DQ667322.1
S. dracocephaloides	96.41%	590/612 (%96)	DQ667265.1
S. bucharica	96.25%	590/613 (%96)	KU563794.1
S. cabulica	96.07%	587/611 (%96)	KJ584189.1
S. garipensis	95.94%	591/616 (%96)	DQ667281.1
S. quezelii	96.24%	589/612 (%96)	KU563808.1
S. huberi	96.09%	590/614 (%96)	KU563800.1
S. ballsiana	96.08%	589/613 (%96)	KU563792.1
S. wiedemannii	96.08%	589/613 (%96)	KU563817.1
S. recognita	96.08%	588/612 (%96)	KU563809.1
S. maymanica	95.93%	589/614 (%96)	KU563805.1
S. blepharochlaena	95.76%	587/613 (%96)	KU563793.1
Nepeta cataria	86.98%	521/599(87%)	AJ515313.1

Table 5. The ITS sequences of *S. tchihatcheffii*

CCGCGAACACGTGTCTAACACCAACCGACGGTGCATGGCGTGGGGGCGACCCCCGTCCTGTTCCCGTCACCCCCGCCC
GCGTGTTCCCATCGGGTCACGTCGGGCGGGCTAACGAACCCCGGCGCGGAATGCGCCAAGGAAAACAAAACGAAGCA
TCCTCCCCCCCGCGCCCCGTTCGCGGAGCGTGCGGGGGCGTCGGATGTCTATCAAATGTCAAAACGACTCTCGGCAACG
GATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCAT
CGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCC
CCCCCTCCGTGCGCGCAGCGCCCGTTGTGGGGGCGGATATTGGCCTCCCGTGCCCCCCCGGCGTGCGGCTGGCCCAA
ATGCGATCCCTCGGCGACTCATGTCACGACAAGTGGTGGTTGAACAACTCAATCTCGCGCGCCGTCGTGCCACTGCG
TCGTCCGCTTGGGCATCCATGAACGACCCAACGGTGCCGGTGCCCCGCAGCACCAACCTTCGACCGCGACCCCAGG
TCAGGCGGGATTACCCGCTGAGTTTAAGCATATCAATAAGCGGAGGA

Total Base Count: 666

In order to determine the molecular similarity of *S. tchihatcheffii*, sequence analysis of the ITS1-ITS4 gene regions was performed. When we look at the results of DNA sequence analysis, it is shown that the sequence analysis of *S. tchihatcheffii* (KU563814.1) and *S. cedronella* (KU563797.1) ITS region has the highest similarity rate.

According to molecular genetic analyses included in the study by Will and Claben-Bockhoff (2017), the endemic species *S. tchihatcheffii* belongs to the evolutionary lineage Subclade I-D within the main group Clade I and shows close kinship with important species such as *S. officinalis*. Similarly, the endemic species *S. albimaculata, S. potentillifolia, S. nydeggeri, S. pisidica, S. cedronella, S. adenophylla*, and *S. heldreichiana* show close kinship in the phylogenetic tree. These seven endemic species are included in group A of *Salvia* in the Flora of Turkey (except for *S. nydeggeri*) (Davis, 1982). This suggests that these endemic taxa share a similar genetic history and undergo evolutionary processes specific to the Anatolian geography.

A study using the RAPD (Randomly Amplified Polymorphic DNA) technique on four endemic *Salvia* species (*S. crypthanta, S. cyanescens, S. tchihatcheffii, and S. wiedemannii)* belonging to the genus *Salvia*, which is defined as a polyphyletic genus, revealed a very high polymorphism rate of 98.7% among the species. In the dendrogram, *S. tchihatcheffii* was found to be genetically closest to *S. wiedemannii* and classified in the same main cluster. These two species, with similar similarity rates, are grouped in the Flora of Turkey (Davis, 1982; Sözen & Yücel, 2015). In another study, it was observed that the chromosome number

of *S. tchihatcheffii* is 2n = 18 (Ozkan, 2006).

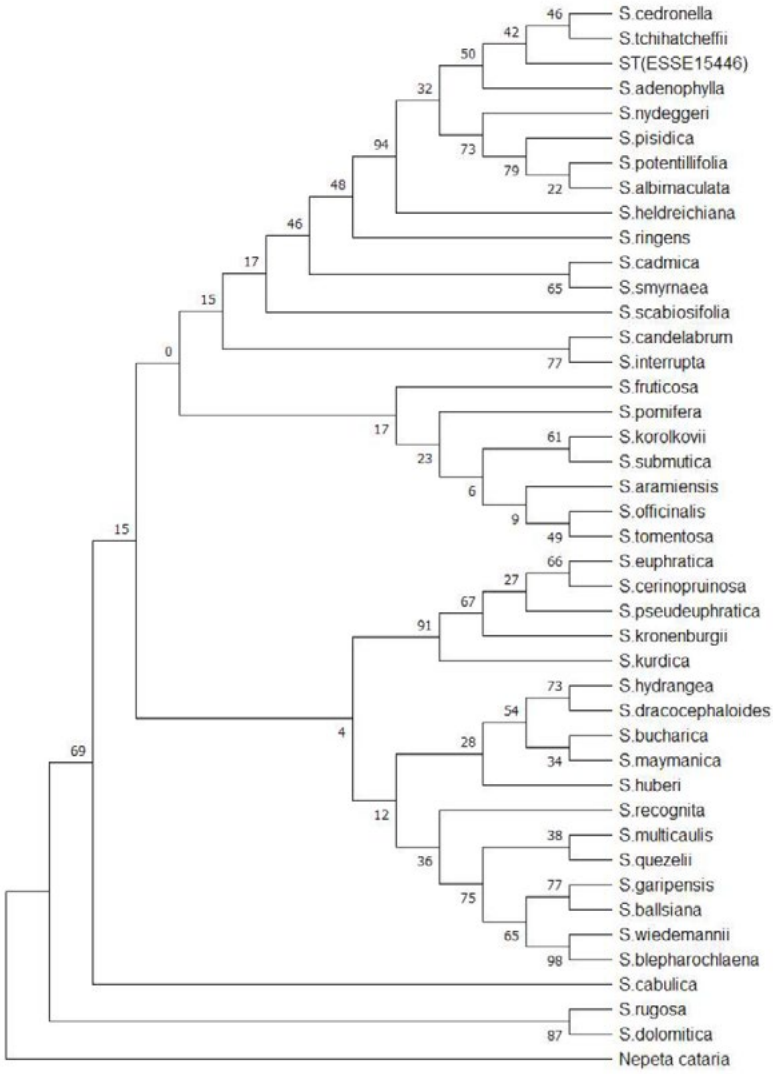

Figure 4. A phylogenetic tree constructed based on the ITS base sequences of taxa

Results of essential oil

The chemical compounds of the essential oils of *S. tchihatcheffii* were analyzed by the gas chromatography technique and shown in Table 6.

According to the findings, the essential oil yield of *S. tchihatcheffii* is high. When GC and GC/MS analyses of the essential oil were examined, 34 chemical compounds were identified for 99.4% of the essential oil of *S. tchihatcheffii*. According to the analysis results, the main component of *S. tchihatcheffii* is camphor, at 23.3%, followed by 1,8-cineol at 15.3% and β-pinene at 11%.

The genus *Salvia* is known to be rich in essential oils. In a study on *S. tchihatcheffii,* 20-23% camphor and 16% 1,8-cineole were found as the major components in its essential oil (Baser, 2002). The 23.3% camphor and 15.3% 1,8-cineole results in this study also support this research.

In another study, the seed oil composition of 12 species of the *Salvia* genus was analysed. Linoleic acid was found as the major component of *S. tchihatcheffii* with 44.2% (Azcan et al., 2024).

In another study, essential oils obtained from *Salvia* by hydrodistillation were analyzed by GC-MS. Camphor was identified as the main component in *S. tchihatcheffii*. When the enantiomeric distribution of this component was analyzed, the oil yield was

0.6% and the relative percentage was 23.3%. The other major components of these species are *α*-pinene, *β*-pinene, limonene, linalool, and borneol, but their ratios vary among species (Demirci et al., 2002).

Table 6. Components of *S. tchihatcheffii* essential oil

RRI	Compounds	%
1032	***α***-Pinene	4.9
1035	***α***-Tugene	1.3
1076	Camphene	8.8
1118	β-Pinene	11.0
1132	Sabinene	tr
1174	Myrcene	0.6
1203	Limonene	1.3
1213	1,8-Cineole	15.3
1255	γ-Terpinene	1.2
1280	*p*-Cymene	2.4
1290	Terpinolene	tr
1474	*trans*-Sabinene hydrate	0.2
1532	Camphor	23.3
1553	Linalool	0.5
1556	*cis*-Sabinene hydrate	0.2
1565	Linalyl acetate	0.2
1611	Terpinen-4-ol	1.2
1612	β-Caryophyllene	2.2
1617	6,9-Guaiadiene	1.3
1661	alloaromadendrene	0.1
1719	Borneol	3.6
1773	δ-Cadinene	0.3
1776	γ-Cadinene	1.1
1980	Furopelargon A	0.5
2008	Caryophyllene oxide	1.4
2104	Viridiflorol	4.5
2105	Furopelargon B	6.7
2144	Spathulenol	1.6
2187	T-Cadinol	2.2
2250	***α***-Eudesmol	0.2
2257	β-Eudesmol	0.7
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=Caryophylladienol II)	0.2
2392	Caryophyllene-2(12),6-dien-5β-ol (=Caryophyllenol II)	0.4
2931	Hexadecanoic acid	tr
	Total	99.4

RRI: Relative retention indices experimentally calculated against n-alkanes; %: calculated from FID data, tr: Trace (<0.1 %)

Total phenolic content determination

The total phenolic content was investigated using gallic acid, and this method is mainly based on the color reaction. The highest total phenol content was observed in the *n*-hexane extract of *S. tchihatcheffii* (Figure 5.).

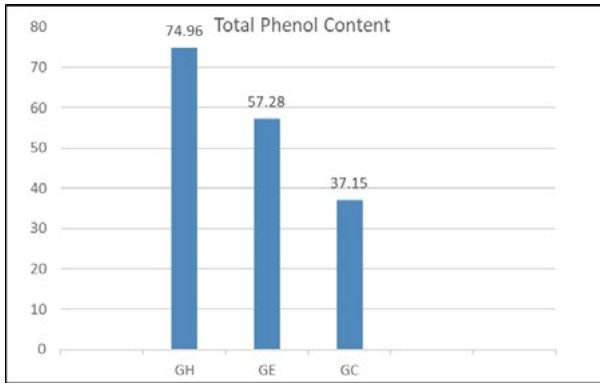

Figure 5. Total phenol mg/g extract (Gallic acid equivalent). G: *S. tchihatcheffii*; H: *n*-Hexane; C: Chloroform; E: Ethanol

Antioxidant activity studies

According to the results of the DPPH method, the highest inhibition value was observed in the *n*-hexane extract of *S. tchihatcheffii.* The results are given graphically in Figure 6. When the antioxidant capacity equivalent to trolox (TEAC) test of the extracts was examined, the lowest TEAC value was observed in the *n*-hexane extracts, and it was seen to be almost the same value in the other extracts (Figure 7.).

Studies have shown that the biological activities of extracts from *Salvia* species are closely related to the secondary metabolite groups they contain. A strong correlation between total polyphenol content and antioxidant activity has been confirmed in aqueous methanol extracts of eleven *Salvia* species (Janicsak et al., 2010). In this context, phytochemical studies on *S. tchihatcheffii* have revealed that the plant contains various classes of secondary metabolites, including sesquiterpenes, diterpenes, triterpenes, steroids, flavonoids, and phenolic compounds (LOTUS; Topcu et al., 1997). They also reported that the methanol extract of *S. tchihatcheffii* (89.95% inhibition) showed much higher activity in the DPPH radical scavenging test compared to the ethyl acetate extract (12.92% inhibition), and that this was related to the high amount of phenolic acid in the methanol extract, and that rosmarinic acid was a major component in the extract (Orhan et al., 2012). When these findings are compared with our study, it is evident that phenolic compounds and flavonoids are the primary sources of antioxidant activity in extracts obtained with polar solvents, whereas diterpenoids are the primary sources in those obtained with non-polar solvents (Topcu et al., 1997; Orhan et al., 2012). However, the high total phenol content and antioxidant capacity found in the *n*-hexane extract, a more nonpolar solvent, in our current study, suggest that the nonpolar compounds of this species may also possess significant antioxidant potential. Detailed content analyses of *S. tchihatcheffii* are necessary to clarify this situation and determine which nonpolar compound groups are responsible for this activity.

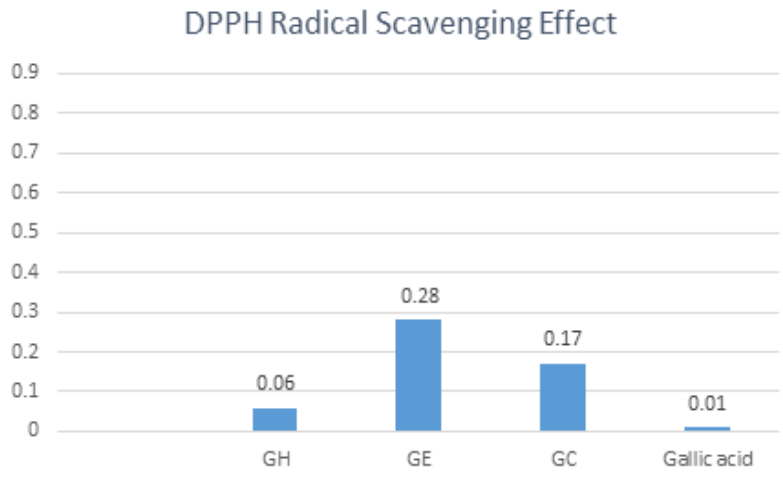

Figure 6. DPPH radical scavenging effect IC_{50} mg/mL. G: *S. tchihatcheffii*; H: *n*-Hexane; C: Chloroform; E: Ethanol

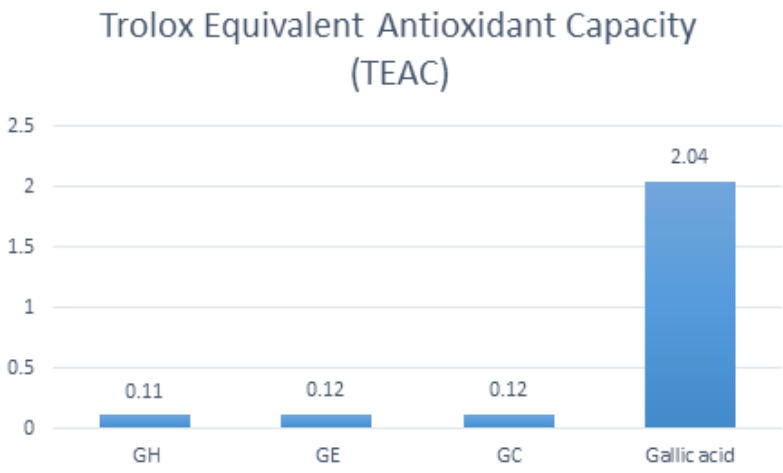

Figure 7. Antioxidant capacity equivalent to mM Trolox at 1 mg/mL concentration. G: *S. tchihatcheffii;* H: *n*-Hexane; C: Chloroform; E: Ethanol

Antimicrobial activity studies

Antibacterial and antifungal effects of *S. tchihatcheffii* extracts prepared with 3 different solvents were investigated. Antimicrobial activity findings for *S. tchihatcheffii* extracts are displayed in Tables 7 and 8. The *n*-hexane and chloroform extracts demonstrated the lowest minimum inhibitory concentration (MIC) values against *Candida* species, particularly exhibiting strong efficacy against *C. utilis* and *C. parapsilosis*. In contrast, the ethanol extract displayed the weakest antimicrobial activity across all *Candida* strains. The antimicrobial effects of the extracts on bacterial species were considerably less pronounced than those observed for *Candida* species. *Escherichia coli* and *Pseudomonas aeruginosa* exhibited complete resistance to all extracts, whereas *Salmonella typhimurium* was identified as the most susceptible bacterium among those tested.

It is an important issue that many pathogenic microorganisms have gained resistance to antibiotics

from the past to the present. The first sources used in antibiotic research are plants, which are considered natural resources. For this reason, it is important to investigate the antimicrobial activity of various plant extracts (Tan et al., 2014-2018). While the components of the plants play an important role in the evaluation of antimicrobial effect, it is seen that the solvents used have an equally important effect. In a study conducted on 30 *Salvia* species growing in Anatolia, it was determined that the antibacterial efficacy of aqueous extracts prepared by infusion and decoction methods was weak, whereas extracts obtained with petroleum ether, chloroform, and ethanol exhibited significantly higher antibacterial activity, particularly against the Gram-positive bacterial species *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA). The chloroform and petroleum ether extracts of *Salvia sericeo-tomentosa* var. *sericeo-tomentosa* demonstrated remarkably potent activity against the standard *S. aureus* strain, with minimum inhibitory concentration (MIC) values of 9.8 µg/mL and 39.6 µg/mL, respectively. Regarding ethanol extracts, *S. sericeo-tomentosa* var. *hatayica and S. albimaculata* were found to be effective against both *S. aureus* and MRSA strains, with an MIC value of 312.5 µg/mL for each. In aqueous extracts, no antibacterial effect was observed in the vast majority of the species studied, even at concentrations below 5000 µg/mL. Only *S. aramiensis* and *S. albimaculata* exhibited limited antibacterial activity, with MIC values of 625 µg/mL against *S. aureus* and 312 µg/mL against MRSA (Tan et al., 2014-2018).

The antibacterial potency of *Salvia* extracts varies considerably depending on environmental factors such as the climate and soil conditions in which the plant grows, as well as the structure of the phenolic compounds (e.g., kaempferol, rosmarinic acid) contained in the extracts. Although sage has the potential to be a strong natural antibacterial source, this potency is also specific to the microorganism (Masotti et al., 2003; Tohma et al., 2016). In a study conducted on 11 *Salvia* species of Iranian origin, it was determined that methanol extracts of *S. eremophila, S. limbata, S. santolinifolia,* and *S. sclarea*, in particular, inhibited the growth (MIC in the range of 0.31-5 mg/mL) of all tested Gram-negative (*E. coli, K. pneumoniae, S. typhi*) and Gram-positive (*S. aureus, S. epidermidis, B. subtilis*) bacteria and showed the highest activity (Firuzi et al., 2013).

Plants of the genus *Salvia* generally exhibit significant antimicrobial activity against microorganisms due to their rich content of secondary metabolites such as volatile oils, diterpenoids, triterpenoids, and phenolic compounds. The strength of this activity varies greatly depending on the plant species and the solvent used in extraction (Demirci et al., 2002; Firuzi et al., 2013; Tohma et al., 2016; Jassbi et al., 2016).

Table 7. Anticandidal effect results of the extracts (MIC, µg/mL)

Microorganisms	St-H	St-C	St-E	S1 µg/mL	S2 µg/mL
***Candida albicans* ATCC 10231**	125	125	250	0.25	0.06
***Candida utilis* NRRL Y-900**	60	30	125	0.125	0.25
***Candida albicans* ATCC 90028**	125	125	250	0.125	0.125
***Candida tropicalis* ATCC 750**	60	60	125	1	0.125
***Candida parapsilosis* ATCC 22019**	60	30	125	0.25	0.125
***Candida krusei* ATCC 6258**	60	60	125	0.5	0.25

St: *S. tchihatcheffii*; H: *n*-Hexane; C: Chloroform; E: Ethanol; S1: Amphotericin-B, S2: Ketoconazole

Table 8. Antibacterial activity results of the extracts (MIC, µg /mL)

Microorganisms	St-H	St-C	St-E	S1 µg/mL
***Escherichia coli* NRRL B-3008**	-	-	-	2
***Staphylococcus aureus* ATCC 6538**	1000	1000	-	1
***Pseudomonas aeruginosa* ATCC 27853**	-	-	-	2
***Salmonella typhimurium* ATCC 13311**	500	500	1000	2
***Serratia marcescens* NRRL B-2544**	1000	-	-	8
***Klebsiella pneumoniae* NCTC 9633**	1000	-	-	8

St: *S. tchihatcheffii*; H: *n*-Hexane; C: Chloroform; E: Ethanol; S1: Chloramphenicol

CONCLUSION

In this study, morphological and anatomical characters of *S. tchihatcheffii*, an endemic species collected from Eskişehir, Türkiye, were investigated. The results were compared with literature data. The kinship relationships between the species belonging to the genus *Salvia* were examined, and *S. cedronella* was found to be the closest species.

Salvia genus is known to be rich in essential oils. Therefore, much research has been carried out on this genus. In this study, the main component of the essential oil was determined as 23.3% camphor and 15.3% 1,8-cineole as a result of GC-GC/MS analysis. The *Salvia* genus has attracted special attention as a source of natural antioxidants. According to the literature data, it is seen that the results vary according to the type of plant and the solvents used. According to the results of Total Phenol, DPPH, and ABTS assays, the best result was seen in the *n*-hexane extract of *S. tchihatcheffii*. The extracts showed low antimicrobial activity.

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AUTHOR CONTRIBUTION STATEMENT

Investigation: G.Ç., F.T., Y.B.K.; Methodology: G.Ç., Y.B.K., G.İ.; Conceptualization: G.Ç., F.T., Y.B.K.; Validation: G.Ç., Y.B.K., G.İ.; Writing – Original Draft: G.Ç., F.T., Y.B.K.; Writing – Review And Editing: G.Ç., F.T., Y.B.K.; Visualization: G.Ç., F.T., Y.B.K.; Supervision: Y.B.K., G.İ.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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