Research Article

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# Evaluation of Antimicrobial Activity of Three Endemic *Salvia* Species Growing in Turkey

## Türkiye'de Yetişen Üç Endemik *Salvia* Türünün Antimikrobiyal Aktivitesinin Değerlendirilmesi

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**ABSTRACT**

The ethanol extracts obtained from the aerial parts of three *Salvia* species (*S. hypargeia*, *S. huberi* and *S. dichroantha*) which are endemic to Turkey were tested for their antibacterial and antifungal activities against two Gram (+) bacterial strains [*Staphylococcus aureus* (ATCC 25925), *Bacillus subtilis* (ATCC 6633)]; three Gram (-) bacterial strains [*Escherichia coli* (ATCC 25923), *Acinetobacter baumannii* (ATCC 02026), *Aeromonas hydrophila* (ATCC 95080)] and *Mycobacterium tuberculosis* H37Rv, and three fungal strains (*Candida glabrata* ATCC 90030, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750) by using the broth microdilution method. All tested plant extracts showed significantly antibacterial activity against *A. baumannii* (ATCC 02026) (62.5 µg/mL MIC value) when compared to reference drug Ampicillin (125 µg/mL MIC value). The ethanolic extract of *S. huberi* was found the most effectiveness extract against *M. tuberculosis* H37Rv with 0.97 µg/mL MIC value when compared to reference antimycobacterial agents Isoniazid and Ethambutol (0.97 µg/mL and 1.95 µg/mL MIC values, respectively).

**Keywords:** *Salvia* species, antimicrobial activity, plant extract, broth microdilution method

**ÖZET**

Türkiye'ye endemik olan üç *Salvia* türünün (*S. hypargeia*, *S. huberi* ve *S. dichroantha*) topraküstü kısımlarının etanollü ekstresi antibakteriyel ve antifungal aktiviteleri için iki Gram (+) bakteri suşu [*Staphylococcus aureus* (ATCC 25925), *Bacillus subtilis* (ATCC 6633)]; üç Gram (-) bakteri suşu [*Escherichia coli* (ATCC 25923), *Acinetobacter baumannii* (ATCC 02026), *Aeromonas hydrophila* (ATCC 95080)] ve *Mycobacterium tuberculosis* H37Rv, ve üç fungal suşa *(Candida glabrata* ATCC 90030, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750) karşı broth mikrodilüsyon yöntemi kullanılarak test edildi. Referans ilaç Ampisilin (125 µg/mL MİK değeri) ile karşılaştırıldığında test edilen tüm bitki ekstreleri *A. baumannii* (ATCC 02026) (62.5 µg/mL MİK değeri) suşuna karşı belirgin antibakteriyel aktivite gösterdi. *S. huberi* türünün etanol ekstresi 0.97 µg/mL MİK değeri ile referans antimikobakteriyel ajanlar İzoniazit ve Etambütol (0.97 µg/mL ve 1.95 µg/mL MİK değerleri, sırasıyla) ile karşılaştırıldığında *M. tuberculosis* H37Rv suşuna karşı en etkili ekstre olarak bulundu.

**Anahtar kelimeler:** *Salvia* türleri, antimikrobiyal aktivite, bitki ekstresi, broth mikrodilüsyon yöntemi

## 1. Introduction

The genus *Salvia* L. (sage) is one of the largest members of the family Lamiaceae (formerly Labiatae) and represented by approximately 1000 species distributed in various regions of the world [1-6] particularly in North and West Asia (especially in Turkey, Russia and Iran), Central and South America, East Asia (especially


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in Japan and China), Europe and Africa [7]. Because of occurring a larger range of primitive morphological types, Soviet Central Asia and Afghanistan are speculated to be the center of origin of the *Salvia* [2]. Anatolia is one of the centers of diversity for *Salvia* in the Old World [7] and the genus comprises 100 species in Turkish flora, 53 (53%) of which are endemic [3, 8-11].

The genus name "sage" comes from the Latin word *salvare* explained as "to heal or to be safe and unharmed" and then in French the name was translated to *sauge* (sage) and in Old English the name was translated to *sawge* [2]. In the Middle Ages and Ancient Romans times, a saying known as: "*Cur moriatur homo cui Salvia crescit in horto?*" meaning "Why should a man die whilst sage grows in his garden?" emphasizes the effect of *Salvia officinalis* L. (Common sage) at that time [2]. Species of *Salvia* have been widely used as a folk medicine for the treatment of menstrual disorders [12-14], stomach ailments [1, 15], common cold [1, 12, 13, 15], bronchitis, tuberculosis [2, 13], cough, chest troubles, respiratory and pulmonary ailments [13], wounds, liver and rheumatism pains [15], malaria, microbial infections, inflammation, cancer, loss of memory [2], hemorrhage and hepatitis [14] since ancient times. Decoction of the aerial parts (leaves-branches) of the *Salvia* species is described as abortifacient to induce act of the urine and the menstrual flow in "De Materia Medica" by Dioscorides [6]. Additionally, infusion of the aerial parts of the genus is used as a haemostatic, tonic, antiseptic, carminative, astringent, diuretic and spasmolytic [9]. In Turkish folk medicine, *Salvia* which is known as "adaçayı" has been used as antiseptic [16-18], antibacterial [18], stomachic, hemostatic [12], stimulants [16], diuretic, spasmolytic, carminative [12, 17, 18], and in the treatment of sore throats [9, 12], colds [9, 17], cough [17] and wounds [16, 18]. Other uses of *Salvia* species and their essential oils were reported as herbal tea [9-11, 15], flavouring agents in perfumery and cosmetic formulations [3, 16, 19], food preservatives [20] and aromatherapy [2].

*Salvia* species are very rich in biologically active secondary metabolites [4] especially polyphenolic compounds [10]. Phytochemical screening showed that species of the genus contain flavonoids [4, 17, 19] (flavones are common than flavonols [17]), monoterpenes [19], diterpenoids [12, 18, 19] (especially abietane-type diterpenes [12, 17]), triterpenoids [12, 17, 19], phenolic acids [17]. Additionally, the essential oils of *Salvia* species are rich in monoterpene hydrocarbons, oxygen-containing monoterpenes and oxygen-containing sesquiterpenes [2].

Up until now, several *Salvia* species have been examined for biological activities including antioxidative [2, 5, 10, 20], immunomodifying, antiproliferative, antineurodegenerative [20], antiinflammatory [2, 5, 12, 20], cardioprotective [12, 20], cytotoxic [5, 12, 18, 21], antiviral [5, 12], antibacterial [12, 18], antifungal [5, 18], antituberculous [12], antiplasmodial, insecticidal [2], antiprotozoal [17], acetylcholinesterase (AChE) and butylcholinesterase (BuChE) [19].

For many years human health has been threatened by pathogen infections and nowadays drug-resistant pathogens and infections such as methicillin-resistant *Staphylococcus aureus* [5] and multidrugresistant tuberculosis (MDRTB) [22] are also another serious problem despite of the abundance of antimicrobial agents [5, 22]. Because of developing resistance and unwanted side effects, more efficient and safer agents with new approaches for management of microbial infectious causing by various pathogens are needed to improve [5]. Plants are rich source of potential new therapeutic agents for alternative treatment of various diseases [23]. Literature data have indicated that there is no study on antimicrobial activity of ethanolic extract of three *Salvia* species (*S. hypargeia* Fisch. & C.A. Mey., *S. huberi* Hedge and *S. dichroantha* Stapf) which are endemic to Turkey. Therefore, the aim of the study was to evaluate antibacterial and antifungal effects of ethanolic extracts obtained from aerial parts of three endemic *Salvia* species against two Gram (+) bacterial strains [*Staphylococcus aureus* (ATCC 25925), *Bacillus subtilis* (ATCC 6633)]; three Gram (-) bacterial strains [*Escherichia coli* (ATCC 25923), *Acinetobacter baumannii* (ATCC 02026), *Aeromonas hydrophila* (ATCC 95080)] and *Mycobacterium tuberculosis* H37Rv, and three fungal strains (*Candida glabrata* ATCC 90030, *Candida parapsilosis* ATCC 22019, *Candida tropicalis* ATCC 750).

## 2. Material and Methods

### 2.1. Chemicals

Ethanol, Dimethyl sulfoxide (DMSO), Fluconazole (Sigma, F8929), Isoniazid (Sigma, I3377), Ethambutol (Sigma, E4630), RPMI 1640 Medium (Sigma, R6504), resazurin sodium salt powder (Sigma R7017), 3-(N-morpholino)-propanesulfonic acid (MOPS, Sigma, M1254) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Middlebrook 7H9 broth, and casitone, glycerol and oleic acid-albumin-dextrose-catalase were purchased from Becton Dickinson (Sparks, MD, USA). The all samples and solutions were prepared by using distilled water and freshly prepared solutions were used for the experiments.

### 2.2. Microbial strains

Bacterial strains, *S. aureus* (ATCC 25925), *E. coli* (ATCC 25923), *B. subtilis* (ATCC 6633), *A.baumannii* (ATCC 02026), *A. hydrophila* (ATCC 95080) and fungal strains, *C. glabrata* ATCC 90030, *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 750 were obtained from the Refik Saydam Hıfzıssıhha Institute, Ankara, Turkey.

*M. tuberculosis* H37Rv was acquired from the Refik Saydam National Public Health Agency, National Tuberculosis Reference Laboratory, Ankara, Turkey.

### 2.3. Plant materials

Plant materials were collected from their natural habitats in different regions of Turkey and identified and confirmed by Dr. Ahmet Kahraman, Department of Biology, Faculty of Arts and Science, Uşak University (Uşak, Turkey). The dried voucher specimens were deposited in Plant Systematics and Phylogenetics Research Laboratory at Uşak University (Table 1).

### 2.4. Extraction procedure

Air dried aerial parts of plant materials were powdered and macerated three times with ethanol (500 mL of solvent per 100 g of plant material) at room temperature. After filtration through Whatman No.1 filter paper, solvents were evaporated at 35-40°C under pressure by using vacuum evaporator (Heidolph-Rotar TLR 1000) and the plant extracts were stored in dark at 4°C until use.

### 2.5. Antimicrobial assays

The broth microdilution method was used for antibacterial [24] and antifungal [25-27] activities and the resazurin microtitre assay (REMA) plate method was used for antimycobacterial activity [22].

### Antibacterial assay

Antibacterial activities of plant extracts were tested against two Gram (+) bacterial strains: S. *aureus* (ATCC 25925), *B. subtilis* (ATCC 6633); three Gram (-) bacterial strains: *E. coli* (ATCC 25923), *A. baumannii* (ATCC 02026) and *A. hydrophila* (ATCC 95080). The plant extracts dissolved in DMSO to prepare stock solutions of the each tested extract then diluted in Mueller-Hilton broth to get an initial concentration of 2000 µg/mL. Ampicillin was used as a reference antibacterial agent. Further dilutions of the each tested extract and ampicillin were prepared at the different concentrations (2000, 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.8, 3.9, and 1.9 µg/mL). The microbial growth effects of tested solvent was determined with a control test containing inoculated broth. DMSO was added at the same dilutions which were chosen for the tested plant extracts, and the results showed that tested solvent had no effect on microbial growth. MIC (the minimal inhibitory concentration) values of each extract were determined in duplicate tests [24].

### Antimycobacterial assay

The resazurin microtitre assay (REMA) plate method was used for antimycobacterial assay [22].

1. Culture medium: 7H9-S medium was used with Middlebrook 7H9 broth containing 0.1% casitone, 0.5% glycerol and 10% oleic acid-albumin-dextrose-catalase for the REMA plate method.

2. Resazurin reagent: The resazurin reagent was prepared from resazurin sodium salt powder. A working solution was done at 0.01% (w/v) concentration in distilled water and filtered to make steril using 0.22 µm membrane filter (Ministar, Sartorius Stedim Biotech GmbH, Goettingen, Germany); the working solution was kept at 4°C for up to 1 week.

**Table 1.** List of plant materials, their origins, voucher references, collecting times and percentage yields of tested extracts.

| Taxon | Locality | Altitude (m) | Collecting time | Voucher references | Phytogeography | Yields (%) |
|---|---|---|---|---|---|---|
| *Salvia hypargeia* Fisch. & C.A.Mey. | Sivas province, between Akdağmadeni and Sarkışla, rockys areas 39.58464°N, 36.20585°E | 1386 | 24.06.2015 | A.Kahraman 2112 | Irano-Turanian element | 8.4 |
| *Salvia huberi* Hedge | Erzurum province, Uzundere to Yusufeli, rocky areas including *Artemisia* sp. 40.59719°N-41.61170°E | 1006 | 25.06.2015 | A.Kahraman 2100 | Irano-Turanian element | 6.18 |
| *Salvia dichroantha* Stapf | Kayseri province, Sarız, near Yukarı Kısrak village, roadsides. 38.26398°N-36.40960°E | 1676 | 10.07.2013 | A.Kahraman 1658 | Irano-Turanian element | 10.96 |

3. The REMA plate method: The REMA plate method was applied in duplicate according to Nateche et al. [22] with some modifications. As reference drugs Ethambutol and Isoniazid and as the standard strain *M. tuberculosis* H37Rv were chosen. Stock solutions of the plant extracts and reference agents were made in DMSO at 1000 µg/mL concentration. 0.22 µm membrane filters were used for filtration of these prepared solutions. In a 96-well microtitre plate, two-fold dilution series of all solutions were prepared by using 100 µL 7H9-S. The concentration ranges between 250-0.12 µg/mL were tested. A growth control with no antibiotic and a sterility control without inoculum were added in each plate. The H37Rv inoculum was prepared by resuspending a loopful of the Lowenstein-Jensen culture medium in a tube containing 5 mL 7H9-S medium with several glass beads. For 2 min. the tube was vortexed and then waited 30 min. for allowing to form sediment. The supernatant was passed to a second sterile tube and the turbidity adjusted to match a McFarland standard No. 1; further dilution of this suspension was 1:20 in 7H9-S. All plates were inoculated with 100 µL suspension and sealed in plastic bags; and incubated at 37°C in a normal atmosphere. After incubation for 7 days, 30 µL resazurin working solution was put into each well; then for 24 h at 37°C the plates were incubated and the results were noted visually. Changes of the resazurin color from blue to pink showed reduction of resazurin and this means bacterial growth. For a positive result, the color changes displaying growth have to be comparable to that seen in the positive growth control. The MIC value was characterized as the lowest solution concentration that prevented full color changes of the resazurin from blue to pink.

**Antifungal assay**

The antifungal activities of the plant extracts were evaluated against three yeast: *C. glabrata* (ATCC 90030), *C. parapsilosis* (ATCC 22019) and *C. tropicalis* (ATCC 750) strains with respect to the standard document (M27-A2) of NCCLS [25] by using the microdilution broth method [26, 27]. Fluconazole was chosen as a reference antifungal agent. Antifungal assay was studied in RPMI 1640 medium which buffered to pH 7.0 with 0.165 M 3-(N-morpholino)-propanesulfonic acid as mentioned in the reference document. A working suspension of standard strains was prepared by a 1:100 dilution followed by a 1:20 dilution of the stock suspension with RPMI 1640 medium. Stock solutions of plant extracts and reference antifungal agent were made in DMSO at 1000 µg/mL concentration. 0.22 µm membrane filter was used for filtration of the solutions. Two-fold dilution series of tested solutions and reference antifungal agent were settled in a 96-well microtitre plate by using 100 µL RPMI 1640 medium. The concentration ranges between 250-0.12 µg/mL were studied. A growth control with no antibiotic and a sterility control without inoculum were added in each plate. Also the working inoculum suspension (100 µl) was put into each plate. The plates were incubated for 48 h at 35°C in ambient air. The MIC value is the lowest concentration of the each tested plant extract inhibits growth of the organism is visually detected.

## 3. Results and Discussion

The extract yields of the studied plants with further details and pictures in their natural habitats were given in Table 1 and Figure 1, respectively. The antimicrobial activities of ethanolic extracts obtained from aerial parts of *S. hypargeia*, *S. huberi* and *S. dichroantha* which are endemic to Turkey were tested against two Gram (+) bacterial strains [*S. aureus* (ATCC 25925), *B. subtilis* (ATCC 6633)]; three Gram (-) bacterial strains [*E. coli* (ATCC 25923), *A. baumannii* (ATCC 02026), *A. hydrophila* (ATCC 95080)] and *M. tuberculosis* H37Rv, and three fungal strains (*C. glabrata* ATCC 90030, *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 750). The antibacterial [24] and antifungal [25-27] activities were performed using the broth microdilution method and antimycobacterial activity [22] was performed using the REMA plate method. The results of the antibacterial and antifungal activities were presented in Tables 2-3. All studied plant extracts inhibited growth of all tested microbial strains.

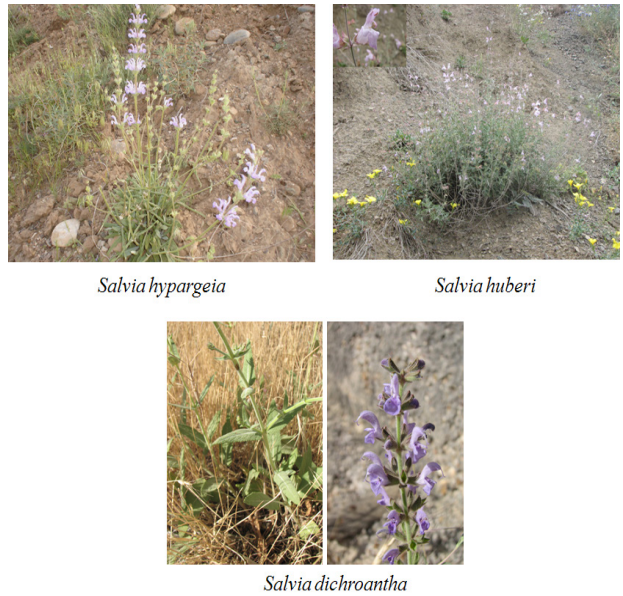


**Figure 1.** Three *Salvia* species (*S. hypargeia*, *S. huberi* and *S. dichroantha*) in their natural habitats.

**Table 2.** The MIC values of the tested plant extracts and reference drugs against bacterial strains (µg/mL).

| Bacterial strains | Code | Plant extracts | | | Reference Drugs | | |
|---|---|---|---|---|---|---|---|
| | | ***S. hypargeia*** | ***S. huberi*** | ***S. dichroantha*** | **Ampicillin** | **Isoniazid** | **Ethambutol** |
| *Staphylococcus aureus* | ATCC 25925 | 125 | 125 | 125 | 31.25 | NT | NT |
| *Bacillus subtilis* | ATCC 6633 | 125 | 125 | 125 | 0.9 | NT | NT |
| *Escherichia coli* | ATCC 25923 | 250 | 125 | 250 | 15.62 | NT | NT |
| *Acinetobacter baumannii* | ATCC 02026 | 62.5 | 62.5 | 62.5 | 125 | NT | NT |
| *Aeromonas hydrophila* | ATCC 95080 | 125 | 125 | 125 | 31.25 | NT | NT |
| *Mycobacterium tuberculosis* | H37Rv | 31.25 | 0.97 | 15.62 | NT | 0.97 | 1.95 |

The MIC values determined in duplicate with deviations within one two-fold dilution. NT: Not tested

**Table 3.** The MIC values of the tested plant extracts and reference drug against fungal strains (µg/mL).

| Fungal strains | Code | Plant extracts | | | Reference Drug |
|---|---|---|---|---|---|
| | | ***S.hypargeia*** | ***S. huberi*** | ***S. dichroantha*** | **Fluconazole** |
| *Candida glabrata* | ATCC 90030 | 62.5 | 62.5 | 62.5 | 3.90 |
| *Candida tropicalis* | ATCC 750 | 62.5 | 62.5 | 62.5 | 15.62 |
| *Candida parapsilosis* | ATCC 22019 | 62.5 | 62.5 | 62.5 | 3.90 |

MIC (the minimal inhibitory concentration) determined in duplicate with deviations within one two-fold dilution.

The results of antibacterial activity studies indicated that the ethanolic extracts obtained from aerial parts of three *Salvia* species (*S. hypargeia, S. huberi* and *S. dichroantha*) showed antibacterial activity with different MIC values between the range of 250 to 0.97 µg/mL against all tested bacterial strains when compared to reference antimicrobial agents (Ampicillin, Isoniazid, Ethambutol). All tested plant extracts had significantly antibacterial activity against Gram (-) bacterial strain *A. baumannii* (ATCC 02026) with 62.5 µg/mL to compare with reference drug Ampicillin with 125 µg/mL MIC values and antimycobacterial activity against *M. tuberculosis* (*S. hypargeia*: 31.25 µg/mL, *S. huberi*: 0.97 µg/mL and *S. dichroantha*: 15.62 µg/mL) to compare with reference drugs Isoniazid and Ethambutol. Moreover, in all studied plant extracts, ethanolic extract of *S. huberi* was found the most effectiveness one against *M. tuberculosis* H37Rv with 0.97 µg/mL MIC value when compared to reference antimycobacterial agents Isoniazid and Ethambutol (0.97 and 1.95 µg/mL MIC values, respectively).

The results of antifungal activity studies demonstrated that the ethanolic extracts of aerial parts of tested *Salvia* species exhibited lower antifungal activity with 62.5 µg/mL MIC value against tested three fungal strains (*C. glabrata, C. parapsilosis* and *C. tropicalis*) when compared to reference antifungal agent Fluconazole.

In previous studies antimicrobial activity of various extracts obtained from different parts of several *Salvia* species were reported against different microorganisms [5, 20, 28, 29].

The antibacterial and antifungal activities of chloroform extract of *S. viridis* L., were investigated against several microorganisms (*Bacillus brevis* FMC 3, *B. megaterium* DSM 32, *B. cereus* FMC 19, *B. subtilis* IMG 22, *Enterobacter aerogenes* CCM 2531, *E. coli* DM, *S. aureus* Cowan 1, *Pseudomonas aeruginosa* DSM 50071, *Micrococcus luteus* LA 2971, *Listeria monocytogenes* Scott A, and *C. albicans* CCM 314 and *C. tropicalis*) by a disk diffusion method. Among all tested microorganisms the plant was only inhibited the growing of *S. aureus* Cowan 1. Moreover, the antifungal effect of the plant was found to be much less than reference antifungal agent Nystatin [28].

The antimicrobial activities of the ethanol extract and essential oil obtained from flowers of *S. hydrangea* DC. ex Benth. were tested against some microorganisms including *E. coli* O157:H7, *L. monocytogenes, B. cereus* and *C. albicans* by agar disc diffusion and serial dilution assays and ethanol extract of the plant which was found more efficient than essential oil exhibited promising antimicrobial activities among all tested microorganisms except *E. coli* [29].

Tohma et al. evaluated antimicrobial activities of ethanol and water extracts of three *Salvia* species (*S. aethiopis* L., *S. brachyantha* (Bordz.) Pobed, *S. microstegia* Boiss. and Bal.) against *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, *B. megaterium* DSM 32), *E. coli* ATCC 11229,

*E. aerugenes* ATCC 13048, *P. aeruginosa* ATCC 9027, *Klebsiella pneumoniae* ATCC 13883, *Yarrowia lipolytica*, *Saccharomyces cerevisiae* and *C. albicans* ATCC 10231, they found that the ethanol extracts of tested plants had broad range antimicrobial activity. *S. microstegia* and *S. brachyantha* were the most active plants against *B. subtilis* [5].

Allimpic et al. studied antimicrobial activity of ethanol extract obtained from aerial part of *S. amplexicaulis* Lam. against some microbial strains including *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *C. parapsilosis* (BEOFB 831 m) and they found that tested plant extract inhibited growth of all tested bacterial strains between 30-40 mg/mL MIC values, but there were no affect detected on growth of tested *Candida* species [20].

There are few studies designed on *S. hypargeia* [3, 11, 12, 18, 30, 31], *S. huberi* [31, 32, 33, 34], and *S. dichroantha* [9, 17, 35] species which were investigated in our study. Karagöz et al. found that the aerial parts of *S. hypargeia* exhibited potent free-radical-scavenging activity with 86.64% [30]. Previous studies indicated the presence of several diterpenoids (hypargenins A-F, microstegiol, aethiopinone, saprorthoquinone, ferruginol [18], 14-deoxycoleon U (=6-hydroxysalvinolone), 5,6-didehydro-7-hydroxytaxodone, salvicanaric acid, demethylcryptojaponol), two triterpenes (lupeol-3-acetate and lupeol) [12] and β-sitosterol, δ-oleanol 3-acetate [18], and also palmitoleic acid (6.4%) and palmitic acid (51.6%) in main component of fatty acid mixture in roots of *S. hypargeia* [12]. Kawazoe et al. studied on methanol extract of the root of *S. dichroantha* and they isolated three novel diterpenes named dichroanal A and B and dichroanone with one known compound, salvinolone [35]. Kırmızıbekmez et al. isolated an aliphatic alcohol glycoside, two megastigmane glycosides (salvionoside B and premnaionoside), two hydroxycinnamic acid derivatives (3-O-methyl-rosmarinic acid and rosmarinic acid), a flavonoid, kaempferol 3,7,4'-trimethyl ether, and sucrose from aerial parts of the plant [17]. Er et al. investigated antioxidant capacity of aerial parts of *S. dichroantha* and antioxidant value was found as 73.855 mg GAE/g [9].

In several studies microbial sensitivities of *Salvia* species were reported. The antimicrobial activity studies on di-and triterpenoid constituents of *Salvia* species growing in Turkey showed that triterpenoids had almost no activity against standard bacteria and a yeast, *Candida albicans* [36]. The species of the genus are rich in polyphenols including flavonoids and phenolic acids and previous studies indicated that phenolic compounds of the *Salvia* species were related to antimicrobial activity [37]. The composition of the studied extracts depend on polarity of used solvent. The polarities of the polyphenols are ranged from non- polar to polar, therefore, broad range of solvents like water, acetone, methanol, ethanol or their mixtures with water were used for extraction [37, 38]. In the present study, ethanol which was dissolving broader range of polar constituents such as phenolics [38] were chosen for extraction. The efficiency of plant extracts also depends on the used part of the studied plants, especially combinations of the plant parts (leaf, root, stem e.g) were showed stronger antimicrobial activity than individual extracts [20]. Gram (-) bacteria are generally more resistant when compared to the Gram (+) bacteria [29]. Moreover, the cell wall of Gram (-) bacteria is more complex than Gram (+) bacteria, outer membranes of both Gram (-) and Gram (+) bacteria are arranged differently and this differences are influenced penetration of macromolecules [20]. In our study, the antibacterial efficiency of all the tested plant extracts on Gram (-) microorganism *A. baumannii* (ATCC 02026) which becomes a serious healthcare problem because of its ability to gain antimicrobial resistance to all classes of antimicrobial compounds [39], and antimycobacterial efficiency of *S. huberi* on *M. tuberculosis* H37Rv which gains resistance to the two most used drugs (Rifampicin and Isoniazid) in the treatment of tuberculosis [22] are indicated that these plants can be promising antimicrobial agents.

## 4. Conclusion

Antimicrobial activity results were in agreement with the above mentioned literature data. *A. baumannii* and *M. tuberculosis* are responsible from serious microbial infections worldwide and also they have ability to acquire resistance to several antimicrobial agents used against them. Therefore, detailed screening on the *Salvia* species investigated our study are required to identify the active compounds that responsible from the antimicrobial activity and further studies are need to compare the activity of these compounds with crude extract. Additionally, further investigations on various microorganisms, animal experiments and toxicological tests are needed to confirm the efficiency of the tested plants for using them as antimicrobial agents in the treatment of infectious diseases.

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