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Antimycobacterial and Antifungal Activities of Selected Four *Salvia* Species

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Abstract: The content of essential oils of endemic *Salvia cilicica* was analyzed by GC-FID and GC-MS techniques. Spathulenol (23.8 %), caryophyllene oxide (14.9 %) and hexadecanoic acid (10.3 %) were identified as the major components in the oil of *Salvia cilicica*. Additionally, in this study ethanol extracts of the aerial parts and essential oils of four *Salvia* species (*S. cilicica, S. officinalis, S. fruticosa, S. tomentosa*), as well as the roots of *S. cilicica* were investigated their antimycobacterial and antifungal activities including infectious diseases. The antimycobacterial activity was analyzed against three *Mycobacterium tuberculosis* (sensitive-, resistant-standard strains and multidrug resistance clinical isolate) strains and the antifungal activity was compared with two dermotophytes (*Microsporum gypseum* and *Trichophyton mentagrophytes* var. *erinacei*) and three *Candida* species by the broth microdilution method. The essentials oils of the four tested *Salvia* species showed high antimycobacterial and antifungal activity (MIC between 0.2-12.5 mcg/mL) in comparison to the aerial parts and root extracts. The antifungal and antimycobacterial potential of the ethanol extracts and essential oils were introduced to determine whether, *Salvia* species can be used in phytotherapy against the yeasts, dermatophytes and *M. tuberculosis*. To the best of our knowledge this is the first study of *S. cilicica* about their antimycobacterial and antifungal activities and chemical composition of its essential oils.

Keywords: *Salvia* species; *Salvia cilicica*; essential oils; antimycobacterial activity; antifungal activity.

1. Introduction

Despite the progress in understanding the growth and control of many pathogens, nearly all the diseases affecting millions of people are still caused by microorganisms. Tuberculosis (TB), a mycobacterial infection, is the most ancient epidemic disease of the world. Even today, it is a serious health problem in many regions of the world and a serious opportunistic disease in HIV/AIDS patients as well. According to the data of WHO, 9 million people had TB and 1.5 million people (360.000 patients were HIV positive) have died from TB in 2013 (1).

There has been an increasing focus on research targeting development of the new drug treatment against TB. However the strain mutations cause drug resistance and the ratio of the multidrug resistance against drugs used in the standard treatment of TB and fungal diseases, such as isoniazid

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(INH), rifampicin (R) and amphotericin B, is increasing. Thus, the searches for novel antimicrobial drugs are essential. The discovery of novel products, useful in the treatment, is a quite challenging and lengthy process. For example, a new antituberculousis agent (Bedaquilin / Johnson & Johnson / TB Alliance) was received an approval with limited indications by FDA after 40 years of research, development and testing in 2012 (2, 3). While the search continues with approximately 10 different drugs against TB, plant-derived natural products may offer potential candidates for novel leads (1, 2).

The *Salvia* (sage) species are potential candidate in this regard, since they have long been used as a frequent component of herbal mixtures to treat TB and a range of microbial infections (4). As seen from the article "Antituberculosis activity of the diterpenic constituents of *Salvia multicaulis* (A. Ulubelen, G. Topcu and C. Bozok-Johansson, 1996)" is a first investigated and patented Anatolian *Salvia* species for the tuberculouse activity (5).

Another important cause of acute and chronic infections is represented by fungi such as recurrent mucosal, cutaneous or nail infections. *Candida* spp. and *Aspergillus* spp. are responsible for the majority (80 to 90%) of fungal infections. *Candida* species cause a lot of infections ranging from non-life-threatening mucocutaneous illnesses to invasive processes that may involve any organ (6-8).

Further, dermatophytosis is frequently associated with people living with HIV/AIDS being 20-40% more than the general population. *Microsporum gypseum (M. gypseum)*, a geophillic dermatophyte frequently isolated from soil, is the most common member of the genus *Microsporum* and may cause tinea capitis and tinea corporis in the immunocompetent hosts. *Trichophyton mentagrophytes* var. *erinacei (T. mentagrophytes* var *erinacei*) is another dermatophyte which causes superficial infections of the skin by invading and parasitizing the non-living keratinized layers. In humans, these infections manifest as highly inflammatory and pruritic eruptions (9, 10).

The genus *Salvia* L. (Lamiaceae) comprises about 900 species world-wide, while it is presented with 89 species and 94 taxa in Turkey, approximately half of which are endemic (11, 12). Anatolia is the major gene center in Asia. *Salvia* species, known as ''adacayi'' in Anatolia, are used in folk medicine for the treatment of a variety of diseases, including infectious diseases. They are used as antiseptics, stimulants, diuretics and for wound healing in Turkish folk medicine and for herbal teas. *Salvia fruticosa* Miller (Syn: *S. triloba* L.) and *Salvia tomentosa* Miller (*S. tomentosa*), which have similar chemical composition and effects with the medicinal species (*Salvia officinalis* L.), are preferred in Turkey beside of *S. officinalis*. The essential oil of *S. fruticosa* is used traditionally as carminative, stomachic, antiperspirant and diuretic (13-15).

Due to several studies indicating antimicrobial, antifungal and antioxidant activities of *Salvia* species, especially *S. officinalis*, similar studies on these species increased gradually all over the world. Additionally, these studies suggested that the hydroxycinnamic acid analogs, flavonoids and diterpenoids contribute to the biological activities of the *Salvia* species (16-19).

S. cilicica Boiss. and Kotschy (SC), an endemic species, has only a limited number of studies in the literature regarding its chemical composition and biological activity. In our previous studies, we have presented the antileishmanial, antioxidant, cytotoxic and antimicrobial (against Gram positive and Gram negative bacteria) activities, and isolation and structure elucidation of the terpenoid compounds from the root extracts which is utilized in traditional medicine (20, 21).

The aim of this study is to examine the antimycobacterial and antifungal potential of the ethanol extracts and EOs derived from *the S. cilicica, S. officinalis, S. fruticosa, S. tomentosa*; to determine if *Salvia* species can be used in phytotherapy against the yeasts, dermatophytes and *M. tuberculosis*, especially MDR-*M. tuberculosis* (MDR-TB, XDR-TB) and to identify the species that provide a convenient agent in phytotherapy against the fungi and *M. tuberculosis*, particularly MDR-*M. tuberculosis* (MDR-TB, XDR-TB).

2. Materials and methods

2.1. *Plant materials*

The aerial parts and roots of *Salvia cilicica* Boiss. and Kotschy (SC) were collected from Adana-Pozanti (Turkey), in September 2011 and identified by Assoc. Prof. Dr. Nur Tan (Istanbul). The

voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 98085). The aerial parts of *S. tomentosa* Miller were gathered from Akaydin /Eskisehir (Turkey), in July 2008, Turkey and the plant materials were identified by Dr. Galip Akaydın and the voucher specimens are kept at the Herbarium of the Faculty of Education, Hacettepe University (HUB 10934) Ankara, Turkey. The aerial parts of *S. fruticosa* Miller were collected from Selcuk-Efes (Turkey) by Hulusi Kütük, in May 2014 and identified by Assoc. Prof. Dr. Nur Tan (Istanbul). The voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 107211). The aerial parts of *S. officinalis* L. were collected from Uludag - Bursa (Turkey) by Önder Mergan, in May 2014 and identified by Assoc. Prof. Dr. Nur Tan (Istanbul). The voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 107212).

2.2. *Extraction and Isolation of essential oil*

The aerial parts (AP) of four *Salvia* species (200 g of each part) and roots of *S. cilicica* were dried at shadow and fresh air and powdered. These were maserated with EtOH (24h) and each extract was lyophilized.

The dried and powdered aerial parts (300 g of each part) of *Salvia cilicica, S. fruticosa, S. officinalis* and *S. tomentosa* were hydro-distillated using a Clevenger type apparatus. The yields as percentage of EtOH extracts were between 1.4-2.0 and EOs 0.4-0.6.

2.3. *Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Flame Ionization Detector (GC-FID)*

GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 mm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min that was kept constant at 220°C for 10 min and followed by elevating the temperature to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. Mass range was *m/z* 35 to 450.

GC-FID analysis

The GC analysis was carried out using an Agilent 6890N GC system using FID detector temperature of 300°C. To obtain the same elution order with GC-MS, simultaneous auto-injection was done on a duplicate of the same column at the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Identification of components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library) (22, 23) and in-house “Baser Library of Essential Oil Constituents” built up by genuine compounds and components of known oils. Additionally, MS literature data (24, 25) was also used for the identification.

2.4. *Biological Assays*

Antimycobacterial Assays (Microdilution Method)

Microdilution method was used according to a standard protocol by Clinical and Laboratory Standard Institute (CLSI) (26, 27). Three strains were tested including each of the following species: *M. tuberculosis* H37Rv ATCC 27294 (Susceptible all antimycobacterial drugs, American Type Culture Collection, USA), *M. tuberculosis* H37Rv ATCC 35838 (resistant to R, American Type Culture Collection, USA) and multi-drug resistant (resistant to INH+R) *M. tuberculosis* that was isolated from patient by Istanbul Faculty of Medicine, Department of Microbiology Laboratory.

Middlebrook 7H9 broth medium (Becton and Dickinson, USA) was used for microdilution method. The medium was adjusted to pH 7.0 at 25°C. Sterility control of each bottle was performed before it was used.

Rifampicin (R) was provided by the Becton Dickinson (BD, USA) as standard lyophilized powders and dissolved in sterile distilled water. *S. tomantosa, S. fruticosa, S. officinalis, S. cilicica* extracts were dissolved in 100 % dimethyl sulfoxide, EOs of *S. tomantosa, S. fruticosa, S. officinalis, S. cilicica* were dissolved in Middlebrook 7H9 with 5 % Tween80 (28) according to CLSI methods (26, 27). The final concentrations were 400 to 3,125µg/mL, and 50 to 0.1µL/mL for extracts and EO's respectively. The critical concentration (1µg/mL) was used for rifampicin.

Preparation of inoculum suspensions of mycobacteria were based mainly according to the CLSI guidelines (26) and described previously (28). The isolates were subcultured on to Löwenstein Jensen medium at 37°C, during 20-25 days. A few colonies from freshly grown *M. tuberculosis* were suspended in Middlebrook 7H9 broth medium to obtain 1.0 McFarland turbidity and then it was diluted ten times using the same medium.

The broth microdilution test was performed by using sterile, disposable microdilution plates (96 U-shaped wells from LP Italiano SPA, Milano, Italy). Rows from 1 to 11 contained the series of drug dilutions in 100 µL volumes and last row (number 12) contained 100 µL of drug-free medium, which served as the growth control. Each well was inoculated on the day of the test with 100 µL of the corresponding inoculum. This step brought the drug dilutions and inoculum size to the final test concentrations given above. The microplates which contained including *M. tuberculosis* were incubated at 37^{o}C until mycobacterial growth was clearly observed in positive control row as white sediment. Mycobacterial growth was confirmed by Ehrlich-Ziehl-Neelsen acid fast stain. For all drugs, the minimal inhibitory concentrations (MICs) was defined as the lowest concentration of the drug which resulted in a complete inhibition of visible growth compared to that one of drug-free growth control (100% inhibition) (26).

Activity of Antifungal

Microdilution method was used according to a standard protocol by CLSI (29-31). Five strains were tested each of the following species: *Microsporum gypseum* NCPF-580 (National Collection of Pathogenic Fungi, Public Health England), *Trichophyton mentagrophytes* var.*erinacei* NCPF 275 (National Collection of Pathogenic Fungi, Public Health England), *Candida parapsilosis* ATCC 22019 (American Type Culture Collection, USA), *C. krusei* ATCC 6258 (American Type Culture Collection, USA), *C. albicans* ATCC 10231 (American Type Culture Collection, USA).

RPMI 1640 broth with L-glutamine without sodium bicarbonate and 0.165 M MOPS buffer (34.54g/L) was used. The medium was adjusted to pH 7.0 at 25°C. Sterility control of each bottle was performed before it was used.

Amphotericin B was provided by the Sigma (Catalog number: A4888) as standard powder and itraconazole was provided by Johnson & Johnson (Johnson & Johnson Sıhhi Malzeme San. ve Tic. Ltd. Şti. Istanbul, Turkey). Amphotericin B, itraconazol and *S. tomentosa, S. fruticosa, S. officinalis, S. cilicica* extracts were dissolved in 100 % dimethyl sulfoxide and EOs of *S. tomentosa, S. fruticosa, S. officinalis, S. cilicica* were dissolved in RPMI 1640 with 5 % Tween80 (32) recommended as CLSI

guidelines (29-31)). The final concentrations extracts were 400 to 3.13µg/mL, amphotericin B and itraconazol were 64 to 0.03µL/mL, for aqueous EO's were 50 to 0.1µL/mL.

Preparation of inoculum suspensions of dermatophytes was based according to the CLSI guidelines (29) and described previously (33). The isolates were subcultured on to potato dextrose agar (PDA) plates at 30°C during 4-5 days. The fungal colonies were covered with 1 mL of sterile 0.85 % saline and suspensions were made by gently probing the surface with the tip of Pasteur pipette. The resulting mixture of conidia and hyphal fragments was withdrawn and transferred to a sterile tube. Heavy particles were allowed to settle for 5-10 min at room temperature; the upper suspension was mixed with a vortex for 15 sec. The turbidity of supernatants was measured spectrophotometrically (Pharmacia, LKB. Ultrospec II) at a wavelength of 530 nm, and transmission was adjusted to 65 to 75 %. These stock suspensions were diluted 1:50 in RPMI medium to obtain the final inoculum sizes, which range from $1x10^3$ to $3x10^3$ CFU/mL.

Preparation of inoculum suspensions of yeasts were based mainly on the CLSI guidelines (30, 31). The colonies of yeasts after 48 h at 35°C of incubation onto Sabouraud dextrose agar (BBL, Sparks, MD, USA) was subcultured to 5 mL sterile saline (0.85 %) and turbidity was adjusted spectrophotometrically at 530 nm 0.5 Mc Farland Standard, and firstly it was diluted 1:50 and then 1:20 in RPMI 1640 in order to obtain a final concentration of 0.5×10^3 to 2.5×10^3 CFU/mL.

The broth microdilution test was performed by using sterile, disposable microdilution plates (96 U-shaped wells from LP Italiano SPA, Milano, Italy). Rows from 1 to 11 contained the series of drug dilutions in 100 µL volumes and last row (row 12) contained 100 µL of drug-free medium, which served as the growth control. Each well was inoculated on the day of the test with 100 µL of the corresponding inoculum. This step brought the drug dilutions and inoculum size to the final test concentrations given above. The microplates which contained dermatophytes were incubated at 28°C during 7 days. The microplates were read visually with the aid to an inverted reading mirror after 7 days for dermatophytes. For all drugs except itraconazole (80% inhibition), the MICs were defined as the lowest concentration of the drug (100% inhibition) which resulted in a complete inhibition of visible growth compared to that one of drug-free growth control (29). For yeasts; a constant volume (100 µL) of the inoculum was added to each microdilution well containing 100 µL of the serial dilution of drugs to reach final concentrations. The microplates were incubated at 35°C for 48 h. For all drugs MICs except itraconazole (80% inhibition) were defined as the lowest concentration showing 100% inhibition of growth (30, 31).

3. Results

The content of the EO of *S. cilicica* was represented in Table 1. In the volatile oil of *S. cilicica* 41 compounds representing 91.3% of the total oil were characterized, with spathulenol (23.8%), caryophyllene oxide (14.9%) and hexadecanoic acid (10.3%) as main components. Additionally, the compounds such as 1,5-epoxysalvial(4)-14-ene (4.4%), caryophyllenol II (3.5%), eudesma-4(15),7-dien-4β-ol (3.2%) were determined.

The extracts and EOs showed various antimycobacterial and antifungal activities. The antimycobacterial activities of the extracts were summarized in Table 2 and antimycobacterial activities of the EOs in Table 3. The experiments were performed with two replications and the results were expressed as average values.

In general, all the extracts and the EOs showed a significant antifungal and antimycobacterial activity via micro dilution tests, but especially the extracts of *S. cilicica* had the highest antifungal activity against *C. parapsilosis* and *T. mentagrophytes* var. *erinacei* among all extracts, while the EOs showed a moderate activity against *M. gypseum*.

The SOA showed the highest antimycobacterial activity with MIC 25 µg/mL against clinical isolate of MDR- *M.tuberculosis* and H37Rv *M. tuberculosis* (ATCC 27294 sensitive) and with MIC 100 µg/mL against H37Rv *M. tuberculosis* (ATCC 35838 R resistant) followed MIC 100 µg/mL by SFA and STA against all three tested *M. tuberculosis* strains. The antimycobacterial activity of the SCA-, and SCR extracts were MIC 400 µg/mL. It is interesting that the SOA indicated good activity against clinical isolate of MDR- *M.tuberculosis*.

Table 1. Main components of the essential oil of *Salvia cilicica.*

RRI	Compounds	%
1535	β-Bourbonene	2.1
1628	Aromadendrene	1.1
1648	Myrtenal	1.4
1670	*trans*-Pinocarveol	1.7
1706	α-Terpineol	1.1
1945	1,5-Epoxy-salvial(4)14-ene	4.4
2008	**Caryophyllene oxide**	**14.9**
2037	Salvial-4(14)-en-1-one	2.0
2071	Humulene epoxide-II	1.1
2130	Salviadienol	1.8
2144	**Spathulenol**	**23.8**
2278	Torilenol	2.6
2287	8,13-Epoxy-15,16-dinor-labd-12-ene	1.6
2289	Oxo-α-Ylangene	1.3
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	1.3
2369	Eudesma-4(15),7-dien-4β-ol	3.2
2389	Caryophylla-2(12),6-dien-5α-ol (=*Caryophyllenol I*)	1.9
2392	Caryophylla-2(12),6-dien-5β-ol (=*Caryophyllenol II*)	3.5
2670	Tetradecanoic acid	1.4
2931	**Hexadecanoic acid**	**10.3**
	<1.0%	8.8
	Total	91.3

** Only the percentages over 1% are indicated in this table*
RRI: Relative retention indices calculated against n-alkanes;
% calculated from FID data

Table 2. The antimycobacterial activities of the ethanol extracts from the root (SCR) and aerial (SCA) parts of *Salvia cilicica*; the aerial parts of *Salvia fruticosa* (SFA), *Salvia officinalis* (SOA) and *Salvia tomentosa* (STA).

The EtOH extracts (400-3.13 µg/mL)	Clinical isolate *M. tuberculosis* (INH+R resistant)	H37Rv *M.tuberculosis* (ATCC 35838 R resistant)	H37Rv *M.tuberculosis* (ATCC 27294 sensitive)
	Microdilution method, MIC µg/mL		
SCA	400	400	400
SCR	400	400	400
SFA	100	100	100
SOA	25	100	25
STA	100	100	100
Rifampicin	> 1 µg/mL	> 1 µg/mL	< 1 µg/mL

MIC = minimum inhibitory concentration

The antifungal activities of the extracts were summarized in Table 4 and of the essential oils in Table 5. The experiments were performed with two replications and the results were expressed as average values.

Table 3. The antimycobacterial activities of the EOs of *Salvia cilicica* (ESC), *Salvia fruticosa* (ESF), *Salvia officinalis* (ESO) and *Salvia tomentosa* (EST).

The essential oils (50-0.1 µL/mL)	**Clinical isolate *M. tuberculosis* (INH+R resistant)**	**H37Rv *M.tuberculosis* (ATCC 35838 R resistant)**	**H37Rv *M.tuberculosis* (ATCC 27294 sensitive)**
	Microdilution method, MIC µL/mL		
ESC	0.4	0.4	0.2
ESF	0.4	0.4	0.1
ESO	0.2	1.6	1.6
EST	0.2	1.6	1.6
Rifampicin	> 1 µg/mL	> 1 µg/mL	< 1 µg/mL

MIC = minimum inhibitory concentration

Table 4. The antifungal activities of the ethanol extracts from the root (SCR) and aerial (SCA) parts of *Salvia cilicica*; the aerial parts of *Salvia fruticosa* (SFA), *Salvia officinalis* (SOA) and *Salvia tomentosa* (STA).

The EtOH extracts (400-3,13 µg/mL)	***Candida parapsilosis* (ATCC 22019)**	***Candida krusei* (ATCC 6258)**	***Candida albicans* (ATCC 10231)**	***Microsporum gypseum* (NCPF 580)**	***Trichophyton mentagrophytes* var. *erinacei* (NCPF 375)**
	Microdilution method, MIC µg/mL				
SCA	50	> 400	400	100	25
SCR	25	400	400	100	12.5
SFA	100	> 400	> 400	50	25
SOA	100	> 400	> 400	100	25
STA	50	> 400	> 400	25	25
Amphotericin B (16-0,03 µL/mL) 100% inhibition	0.5	4	0.5	1	2
Itraconazole (16-0,03 µL/mL) 80% inhibition	0.12	0.5	0.06	0.5	0.5

MIC = minimum inhibitory concentration

Table 5. The antifungal activities of the EOs of *Salvia cilicica* (ESC), *Salvia fruticosa* (ESF), *Salvia officinalis* (ESO) and *Salvia tomentosa* (EST).

The essential oils (50-0,1 µL/mL)	***Candida parapsilosis* (ATCC 22019)**	***Candida krusei* (ATCC 6258)**	***Candida albicans* (ATCC 10231)**	***Microsporum gypseum* (NCPF 580)**	***Trichophyton mentagrophytes* var. *erinacei* (NCPF 375)**
	Microdilution method, MIC µg/mL				
ESC	0.2	3.12	3.12	0.4	0.4
ESF	0.2	3.12	3.12	0.4	0.4
ESO	0.4	6.25	3.12	0.4	0.8
EST	0.8	12.5	6.2	0.4	0.8
Amphotericin B (16-0.03 µL/mL) 100% inhibition	0.5	4	0.5	1	2
Itraconazole (16-0.03 µL/mL) 80% inhibition	0.12	0.5	0.06	0.5	0.5

MIC = minimum inhibitory concentration

The antimycobacterial activity results of the EOs were significant in the antifungal tests. The ESO and EST showed strong antimycobacterial activity (MIC 0.2 µL/mL), especially against MDR-clinical isolate. However, ESC and ESF showed higher antimycobacterial activity in comparison to ESO and EST against resistant (ATCC 35838) and sensitive (ATCC 27294) H37Rv *M. tuberculosis* strains. In general, all EOs showed MIC's ranges between 0.2-0.4 µL/mL against clinical isolate MDR-*M. tuberculosis* and between 0.4-1.6 µL/mL against resistant H37Rv *M. tuberculosis*.

The SCR showed higher antifungal activity against *C. parapsilosis* and *T. mentagrophytes* var. *erinacei* than SCA. The activities of all extracts were weak in comparison to the positive control antifungal agents Itraconazole and Amphotericin B, especially against *C. krusei* and *C. albicans*; but they exhibited good activities against *T. mentagrophytes* var. *erinacei.*

The EOs of all four tested *Salvia* species indicated very high antifungal activity. Some activities of them such as ESC and ESF were remarkable. The ESC and ESF showed higher activity against *C. parapsilosis* (MIC 0.2 µL/mL), *M. gypseum* (MIC 0.4 µL/mL) and *T. mentagrophytes* var. *erinacei* (MIC 0.4 µL/mL) than Amphotericin B and Itraconazole. The antifungal tests demonstrated that all EOs have very good antifungal activity against *M. gypseum* and *T. mentagrophytes* var. *erinacei*.

4. Discussion

There are several previous studies on the antifungal activity of various *Candida* species on various *Salvia* species as well as on the extracts and essential oil of *S. officinalis, S. fruticosa* and *S. tomentosa* (15, 17-19, 28, 34-37). However, the antimycobacterial activity of *S. officinalis* and *S. cilicica* has not been determined before.

One of the important recent studies on *Salvia fruticosa* was published in Turkish Journal of Chemistry by Topcu et al., which covers phytochemical constituents of the extracts as well as essential oil studies of the plant (38).

Askun *et al.* investigated the antimycobacterial activities, besides of the antimicrobial activities, on *S. fruticosa* and *S. tomentosa*. In this study, they exhibited the antimycobacterial activities of the EOs from the aerial parts of five *Salvia* species in Turkey (*S.aucheri* Bentham (endemic), *S. aramiensis* Rech.Fil., *S. fruticosa, S. tomentosa* and *S. verticillata* L. subsp. amasiaca Freyn & Bornm) (39-40).

The aerial parts of 16 *Salvia* species, collected in South Africa and used as folk medicines against various infections, were tested against *M. tuberculosis* as well as the other bacteria. The shown activity against *M. tuberculosis* (MIC<0.50mg/mL) with *S. radula* Benth., *S. verbenaca* L. and *S. dolomitica* Codd was remarkable (41).

The activities of the *S. officinalis'* essential oil and the antifungal agents against *C. albicans, C. parapsilosis, C. krusei* and *C. glabrata* were examined by Badiee et al and the possibility of usage of *S. officinalis* as a natural alternative antifungal agent was exhibited (28). In addition, another study exist in literature, in which the antifungal activities of the EO from *Salvia sclarea* and major essential oil constituents (linalyl acetate and linalool) against 30 clinical isolates yeasts, such *as C. albicans, C. tropicalis, C. krusei, C. glabrata* and *C. parapsilosis*, were investigated. The study results indicated that its essential oil showed stronger activity than the isolated major compounds (42).

Pinto et al were tested the activities against yeasts, filamentous fungi (dermatophytes and phytopatogenic and spoilage species), on *S. officinalis* with macrodilution broth method. The ranges of MICs were identified 1.25- 10 µL/mL against yeasts such as *C. albicans, C. krusei* and *C. parapsilosis*; and, 0.63–2.5 µL/mL against dermatophytes such as *T. mentagrophytes* and *M. gypseum* (35).

Three extracts (water decoction and infusion; methanol: water (80:20)) of this species were determined with disc diffusion method and founded active against some *Candida* species as well as *C. parapsilosis* (37).

Dulger et al presented the antifungal activity of the ethanol extracts from the different parts of an endemic species (*S. tigrina* Hedge & Hub. Mor.) in Turkey, all extracts showed an activity, especially against *Candida albicans (C. albicans), Candida neoformans (C. neoformans)* and *Botrytis cinarea* (32).

Tepe et al present the effectiveness of the essential oil and the different extracts of *S. tomentosa* against the *C. albicans* and *C. krusei* (15). Similar researches and results by Sokovic et al exist on *S. fruticosa*, too (17).

The activity studies on the isolated compounds exist in the literature and are important for determine and comment the activity of plants (5, 43). According the results of one research, camphor, α-thujone, β-thujone, 1,8-cineole, linalool, linalyl acetate, shown good antifungal activity (18). Linalool and linalyl acetate strong activities against various *Candida* species such as *C. albicans*, *C. krusei*, *C. parapsilosis* (42 ~~41~~). Eucalyptol (1.8-cineole) and camphor are well-known chemicals having antimicrobial potentials (44, 45).

Tuberculosis (TB) and fungal diseases, both are require a long period of treatment. During this long treatment period it is possible to develop resistance against the commonly used drugs, or toxicity problems (e.g. Amphotericin B, Bedaquilin toxicity etc) may occur and such problems will often lead to treatment failure. Therefore, the need for new and more effective compounds/drugs is critical.

In our study, the results were different than previous studies. Especially, the EOs have very high antimycobacterial and antifungal activities with different MIC values.

This study presents first time -the chemical composition of the EO of *S. cilicica* and – comparison of its potent antifungal and antimycobacterial activities against three *Salvia* species, which are used traditionally in Anatolian. According to the literature and our knowledge, it is the first study to compare the endemic *Salvia cilicica* with traditionally used other three *Salvia* species regarding their antimycobacterial and antifungal activities. In conclusion our results presented high antimycobacterial and antifungal activities of essential oils of *S. cilicica* and other three *Salvia* species would be the additional treatment solutions of tuberculosis and fungal diseases.

Further studies regarding on pharmacological, animal tests and toxicological investigations are required to provide more conclusive proof of their antimycobacterial and antifungal activities.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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