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Determination of Antimicrobial and Biological Activities of *Salvia sclarea* L. (Lamiaceae) Extracts

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Abstract: Therapeutic plant species of genus Salvia are important and highly recommended medicinal plant, due to their pharmaceutical properties. In the present study, S*alvia sclarea* L. was collected during its flowering stage in 2016, Sarıcakaya (Eskişehir/Turkey) and dried medicinal plant material were macerated with 70% MeOH. The antimicrobial activity of *Salvia sclarea* extracts was determined with minimum inhibitory concentration (MIC) assay against *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 14990, *Enterecoccus faecalis* ATCC 51299, *Bacillus subtilis* NRRL B478, *Escherichia coli* ATCC 35218, *Candida albicans* ATCC 90028 and *Candida krusei* ATCC 6258. The antibiofilm activity of *Salvia sclarea* extracts was determined against *Staphylococcus aureus* ATCC 29213 and *Staphylococcus epidermidis* ATCC 14990 *Candida albicans* ATCC 90028 and *Candida krusei* ATCC 6258. In addition, the preliminary cytotoxicity assessment of extracts was tested with Brine Shrimp Lethality Assay against model organism *Artemia salina* nauplii. As a result, *Salvia sclarea* extract showed remarkably antimicrobial antibiofilm efficacy to all tested microorganisms. The cytotoxicity concentrations of S*alvia sclarea* plant extract also was determined. These results suggest the potential use of the S*alvia sclarea* L. extract as natural medicine in pharmaceutical industry.

Keywords: *Salvia sclarea;* antimicrobial; antibiofilm; cytotoxicity.

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INTRODUCTION

Plant derivates are an important source of natural products and considered as new treatments of various diseases. They have a wide variety of structural and biological attributes. They have played a significant role in traditional medicine of various countries. *Salvia sclarea* L. is an important genus of the *Lamiaceae* family that has approximately 1000 species and represented by nearly 99 species with 58 taxa endemic species in Turkey. *Salvia sclarea* is locally known as "Paskulak" (1,2). S*alvia sclarea* L. general plant view is shown in Figure 1. Turkey is an important country for export and usage of Salvia species in the world (3). Salvia species are used in medicine for the treatment of many popular illnesses such as stomach aches, colds and pain in the throat as herbal tea (4). They possess a number of biological activities including antiseptic, antibacterial (5,6), antibiofilm, antioxidant (7), anti-inflammatory (3,8,9), antiviral (10,11), antitumoral (12) cytotoxic (6,13,14), spasmolitic, anticonvulsant (15) antimycobacterial (4), and carminative activities (16). Nowadays, with the increasing of antibiotic resistance in microorganisms, it became a necessity to find effective alternatives. Therefore, in the current study antimicrobial, antibiofilm and lethality activities of *Salvia sclarea* L. was defined by using different biological methods.

Figure 1: General plant view of S*alvia sclarea* (Photo: Sevim Küçük)

MATERIALS AND METHODS

Plant Materials

S*alvia sclarea* L. was collected during the flowering stage in 2016, Sarıcakaya (Eskişehir/Turkey). Collected plant samples were identified and prepared voucher specimens are kept at the Herbarium of Faculty of Pharmacy of Anadolu University, Turkey (ESSE NO:14701). Plants were dried with medicinal plant materials and macerated with 70% MeOH. Aqueous extracts were evaporated at 80 °C, 90 rpm with a rotary evaporator and crude extract was gained. Then, plant extracts were lyophilized. 50 g of plant extract was obtained as a result. Different concentrations of dried extracts were resuspended in dimethyl sulfoxide (DMSO).

Determination of Minimum Inhibitory Concentration (MIC)

The MICs were defined as the lowest concentrations necessary for the inhibition of growth. Clinical Laboratory Standards Institute (CLSI) microdilution broth methods (17,18) were used. Five different concentrations of *Salvia sclarea* extracts were applied to the *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 14990, *Enterecoccus faecalis* ATCC 51299, *Bacillus subtilis* NRRL B478, *Escherichia coli* ATCC 35218, *Candida albicans* ATCC 90028 and *Candida krusei* ATCC 6258 species by using 96 well plate assay. *Salvia sclarea* extracts were weighed and dissolved in dimethyl sulfoxide (DMSO). Proper concentrations were 3000, 1500, 750, 375 and 187.5 µg/mL. Overnight microorganism cultures were adjusted to McFarland 0.5 standard (approximately 10^8 colony-forming units (CFU)/mL) 100 µL of extract and 100 µL of each microorganism cultures were inoculated to the well plates and incubated at 37 °C, 24 hours. Ketoconazole for yeast species (*Candida albicans* and *Candida krusei*), chloramphenicol for bacteria species (*Escherichia coli, Staphylococcus aureus Staphylococcus epidermidis, Enterecoccus feacalis, Bacillus subtilis*) and ethanol (C_2H_5OH) were used as positive control at 6000, 3000, 1500, 750, 375, 187.5, 93.75, 46.875, 23,437 and 11.718 µg/mL. Sterile distilled water was also used as negative control. After incubation period wells were stained by 20 µL resazurin dye to observe the color difference between dead and living cells.

Biofilm eradication assay (Determination of Minimum Biofilm Eradication Concentration (MBEC))

Microbial biofilms are communities of microorganisms that are embedded in a self-producing matrix, forming on living and nonliving surfaces. The antibiofilm activity of *Salvia sclarea* extract was determined by using the minimum biofilm eradication concentration (MBEC) assay. The eradication effect of *Salvia sclarea* extracts on biofilm formation was evaluated in 96-well plates. Briefly, overnight *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 14990, *Candida albicans* ATCC 90028 and *Candida krusei* ATCC 6258 cultures were adjusted to McFarland 0.5 standard standard (approximately 10^8 colony-forming units (CFU)/mL). 200 µL of each bacteria were inoculated to the well plates and incubated at 37 °C, for 48 hours to form the biofilm at the bottom of the wells. After incubation period, wells were gently washed two times with 100 µL of %0.90 NaCl (physiological saline). 100 µL of *Salvia sclarea* extract (3000, 1500, 750, 375 and 187.5 µg/mL) was added to each well and incubated at 37 °C, 24 hours. Ethanol (C_2H_5OH) and sterile distilled water were used as positive and negative controls. After the incubation period, wells were stained by 20 µL resazurin dye.

Brine Shrimp Lethality test

A 24-h LC_{50} bioassay was performed in a 96 well plate using nauplii of the Brine shrimp *Artemia salina* (19). Commercially sold ROTIFISH *Artemia* mix was used. Eighteen grams of *Artemia* mix was poured into 500 mL of distilled water and incubated 48-52 hours at 30 °C. After the incubation period, larvae were taken and counted. Brine shrimp lethality bioassay was determined with *Salvia sclarea* extract concentrations (3000, 1500, 750, 375, 187.5, 93.75, 46.87, 23.43, 11.71, 5.85 µg/mL) with ten (10) *Artemia salina* larvae in each concetration well. After 24 hours of incubation period, the alive larvae were counted.

RESULTS AND DISCUSSION

Extraction Yield

S*alvia sclarea* L. plant samples were extracted by 70% MeOH extraction method. The extraction yields were 62.5% by weight.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC values of *Salvia sclarea* extracts were determined to be 750 µg/mL for *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 14990, *Bacillus subtilis NRRL B478*,

Escherichia coli ATCC 35218 and 1500 µg/mL for *Enterecoccus faecalis* ATCC 51299. Moreover, 750 µg/mL for *Candida albicans* ATCC 90028 and *Candida krusei* ATCC 6258 was determined. These results indicated that plant extract has strong broad-spectrum antimicrobial activity. All of the MIC values showed that *Enterecoccus faecalis* ATCC 51299 is more resistant than other test micoorganisms. Results are represented in Tables 1 and 2. *Salvia sclarea* plant extract showed equal bacteriostatic activity against test bacteria and yeast strains. Our results are similar to observations that previously reported in the literature, indicating that Gram-positive, Gram-negative bacteria and yeasts showed same activity with same MIC values.

Table 1: MIC values of *Salvia sclarea* plant extract and chloramphenicol against bacteria species.

Test Strains	**MIC values of *Salvia sclarea extract* (µg/mL)**	**Chloramphenicol (µg/mL)**
Staphylococcus aureus ATCC 29213	750	23.43
Staphylococcus epidermidis ATCC 14990	750	NG
Enterecoccus faecalis ATCC 51299	1500	187.5
Bacillus subtilis NRRL B478	750	NG
Escherichia coli ATCC 35218	750	93.75

*NG: No growth.

Table 2: MIC values of *Salvia sclarea* plant extract and Ketoconazole against yeast species.

Test Strains	**MIC values of *Salvia sclarea extract* (µg/mL)**	**Ketoconazole (µg/mL)**
Candida albicans ATCC 90028	750	187.5
Candida krusei ATCC 6258	750	48.875

Biofilm eradication assay (Determination of Minimum Biofilm Eradication Concentration (MBEC))

Antibiofilm studies demonstrated a dose-dependent activity. The antibiofilm activity of *Salvia sclarea extract* was examined by minimum biofilm eradication concentration (MBEC) assay. The MBEC values were determined to be 3000 µg/mL for all test microorganisms. It means that, *Salvia sclarea* extract has same antibiofilm effect against the selected bacteria and yeast species as shown in Table 3. It is very difficult to eradicate and treat microbial biofilms. *Salvia sclarea* plant extract should be considered of great value in treating biofilms due to its minimum biofilm eradication concentrations (MBEC).

Table 3: MBEC values of *Salvia sclarea* plant extract against test microorganisms.

Test Strains	**MBEC values of *Salvia sclarea extract* (µg/mL)**
Staphylococcus aureus ATCC 29213	3000
Staphylococcus epidermidis ATCC 14990	3000
Candida albicans ATCC 90028	3000
Candida krusei ATCC 6258	3000

Brine Shrimp Lethality test

The Brine Shrimp Lethality test results were determined at 3000, 1500, 750, 375, 187.5, 93.75, 46.87, 23.43, 11.71, 5.85 µg/mL concentrations. After 24 hours incubation period, live larvae were counted and the results are shown in Table 4. The Brine Shrimp Lethality test concentrations were determined at 93.75 and 46.87 µg/mL.

Table 4: Lethality values of *Salvia sclarea* plant extract against *Artemia salina* larvae.

***Salvia sclarea* plant extract concentrations (µg/mL)**	**3000**	**1500**	**750**	**375**	**187.5**	**93.75**	**46.87**	**23.43**	**11.71**	**5.85**
Number of *Artemia salina* larvae out of 10	All dead	All dead	All dead	All dead	All dead	6	8	All alive	All alive	All alive

CONCLUSION

Antimicrobial, antibiofilm and cytotoxicity methods are extensively used to investigate the biological activities of natural substances. There are limited data in the literature about the biological activities of *Salvia sclarea* L. plant extracts. The results of this study revealed that *Salvia sclarea* L. plant extract exhibited significant antimicrobial and antibiofilm activity against all tested microorganism strains in vitro. The mechanisms of antimicrobial and antibiofilm activities of natural compounds and extracts are still not fully understood. Previous studies reported that there is a relationship with the chemical composition of the plants and the antimicrobial activities. These chemical components exerted their toxic effect against microorganisms by disruption of membrane integrity (20). The mechanism of these disruption molecules in plants is not accurately determined. In addition, these studies provided an experimental basis of practical application of *Salvia sclarea* plant extract as a natural antimicrobial and antibiofilm agent. The Brine Shrimp lethality test is an important bioassay which is an indication of cytotoxicity, pesticidal effects and various pharmacological and biological activities of natural substances. The cytotoxic effects of plants are principally contributed by the presence of secondary metabolites like alkaloid, glycoside, steroid, tannin and flavonoid in their extract (21). This is also revealed with our results that *Salvia sclarea* L. plant extracts showed cytotoxic activities at some concentrations.

In conclusion, plant extracts of *Salvia sclarea* growing in Turkey, have significant pharmacological importance. Different *Salvia sclarea* plant extract concentrations and microorganism cultures may be used in further studies to obtain better results. And for the determination of cytotoxic activity lower *Salvia sclarea* plant extract concentrations may be used.

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