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Research Article

Study on Antimicrobial and Antibiofilm Activities of *Salvia microstegia* mericarps from Turkish flora

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Abstract

Biofilm-associated infections cause important health problems all over the world. Because of the resistance to antimicrobials and their possible side effects, researchers' attention has been focused on plant extracts and their components. The aim of this study is to evaluate the antimicrobial and antibiofilm potentials of *Salvia microstegia* Boiss. et Bal. mericarps. The antimicrobial activity of ethanolic extract obtained from the mericarps was evaluated using the microdilution method against seven reference bacteria and three standard yeast strains. In addition, the effect of the extract on inhibiting biofilm formation and preformed biofilm of *Pseudomonas aeruginosa* was determined using the crystal violet method. The used extract showed moderate to low antimicrobial activity against tested bacteria and yeasts with minimum inhibitory concentration (MIC) values ranging from 62.5 to 250 µg/mL. Moreover, the extract's potential to inhibit preformed biofilms was found to be greater compared to its potential to inhibit biofilm formation. In conclusion, the tested extract obtained from *Salvia microstegia* mericarps showed antibiofilm potential, however, additional studies involving different strains are needed to reveal its antimicrobial potential in a more holistic perspective.

Keywords: *Salvia microstegia*, mericarp, ethanol extract, antimicrobial activity, antibiofilm activity

Salvia microstegia Merikarplarının Antimikrobiyal ve Antibiyofilm Aktiviteleri Üzerine Bir Araştırma

Öz

Biyofilm ilişkili enfeksiyonlar tüm dünyada önemli sağlık sorunlarına neden olmakatadır. Antimikrobiyallere karşı gelişen direnç ve olası yan etkiler nedeniyle, araştırmacıların dikkati bitki ekstreleri ve onların bileşenleri üzerine yoğunlaşmıştır. Bu çalışmanın amacı *Salvia microstegia* Boiss. et Bal. merikarplarının antimikrobiyal aktivite ve antibiyofilm potansiyellerini değerlendirmektir. Merikarplardan elde edilen etanol ekstresinin antimikrobiyal aktivitesi yedi standart bakteri ve üç standart maya suşuna karşı mikrodilüsyon yöntemi kullanılarak belirlenmiştir. Ayrıca ekstrenin *Pseudomonas aeruginosa*'nın biyofilm oluşumunu inhibe etme ve oluşmuş biyofilmi üzerine etkisi kristal viyole yöntemi kullanılarak belirlenmiştir. Kullanılan ekstre, 62.5–250 µg/mL aralıkta minimum inhibisyon konsantrasyonu (MİK) değerleri ile test edilen bakteri ve mayaları karşı orta ve düşük antimikrobiyal aktivite göstermiştir. Bununla birlikte, ekstrenin önceden oluşturulmuş biyofilmleri inhibe etme potansiyelinin biyofilm oluşumunu engelleme potansiyelinden daha iyi olduğu tespit edilmiştir. Sonuç olarak, *Salvia microstegia* merikarplarından elde edilen ekstrenin antibiyofilm potansiyeli bulunmakla beraber, antimikrobiyal potansiyelinin daha geniş çerçevede ortaya konabilmesi için farklı suşların dâhil edildiği ilave çalışmalara ihtiyaç vardır

Anahtar Kelimeler: *Salvia microstegia*, merikarp, etanol ekstresi, antimikrobiyal aktivite, antibiyofilm aktivite

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1. Introduction

Nowadays, resistance to antibiotics has become a serious global health problem. Increased infection rates due to antimicrobial resistance pose a major problem both for human health and economic costs by increasing death rates and causing more hospitalizations [1]. It is known that microbial biofilms play an important role in the pathogenesis of various infectious diseases, as well as the planktonic forms of microorganisms. Microbial biofilms are communities of microorganisms embedded in their own organic exopolysaccharide matrix that adhere to living and non-living solid surfaces [2]. In addition, biofilm-associated microorganisms cause serious health problems by adhering to a wide variety of surfaces, including permanent medical devices such as catheters, valves, and prostheses [3,4]. Biofilms, which are highly resistant to antibiotics and host immune defenses, are considered an important virulence factor, especially causing persistent chronic and recurrent infections [5]. Biofilm, which are up to 1,000 times more resistant to antimicrobials than planktonic cells, create serious problems in the treatment and severely limit the treatment options [6,7]. The mechanisms underlying biofilm formation and antimicrobial resistance is diverse, such as limited diffusion into the biofilm matrix and reduced antibiotic penetration, type IV secretion systems, the persister phenomenon, and the effect of antibiotic modifying enzymes [8,9]. Antibiotics used in the treatment of biofilm-derived infections cannot completely eliminate the biofilm rather they reduce its amount. For the above reasons, biofilm inhibition is considered the main drug-targeted process for the treatment of various infections [10].

'Traditional medicine', which is considered an important option in the treatment of human diseases, is widely acknowledged in many parts of the world. Many medicinal plants, which also have significant commercial value, are accepted as effective natural health sources for the treatment of various diseases [11,12].

The genus *Salvia* (sages), comprising nearly 1000 species, more than half of which are found in North and South America, is reported as the most species-rich genus of the *Lamiaceae* family [13]. After Mexico, Turkey has the second largest number of *Salvia* species in the world consisting of an approximately 100 species, of which about 53 are endemic [14]. Species of these genus have been traditionally used for the management of common cold, bronchitis, tuberculosis, wounds, malaria, microbial infections, inflammation, cancer, hemorrhage, and hepatitis since ancient times [15]. Because of bioactive constituents (terpenoids, phenolics, essential oils, and fatty acids e.g.) [14,16-18] and medicinal properties (antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and anti-Alzheimer's activities) [19], several *Salvia* species have great importance in pharmaceutical, food, perfumery, and cosmetic industries [19,20]. Herbaceous perennial herb, *Salvia microstegia* Boiss. et. Bal is known as "yağlambaç" in Turkey [21]. Some chemical and activity studies on different parts of the plant were reported in the literature [22,23]. However there is no study on antimicrobial and antibiofilm activities of ethanolic extract obtained from *S. microstegia* mericarps from Turkish flora. Therefore, the purpose of the current study is to examine the antimicrobial and antibiofilm activities of *S. microstegia* mericarps.

2. Material and Methods

2.1. Plant material

Plant materials were collected from their natural habitat between Refahiye-İmranlı, Turkey. The samples were identified/confirmed by Dr. A. Kahraman (Department of Biology, Faculty of Arts and Science, Uşak University, Uşak, Turkey). The dried voucher specimens were deposited in the Plant Systematics and Phylogenetics Research Laboratory, Uşak University.

2.2. Extract preparation

The powdered mericarps were macerated with ethanol (X3, 96 %, 20 mL of ethanol per 1 g of sample) at room temperature and then filtered via Whatman Grade No.1 filter paper. In the next step, the solvent was evaporated using a vacuum evaporator (Heidolph Instruments GmbH & CO. KG, Germany). The obtained extract was stored at 4°C in the dark until use for activity studies [24].

2.3. Antimicrobial activity study

2.3.1. Microbial strains

Seven standard bacterial strains including *Escherichia coli* ATCC 35150, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Klebsiella pneumoniae* ATCC 100031, *Acinetobacter baumannii* ATCC 02026, *Bacillus subtilis* ATCC 6633, and three standard yeast strains including *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 15126, and *Candida parapsilosis* ATCC 90018 were used for antimicrobial activity studies.

2.3.2. Antimicrobial activity

Antimicrobial susceptibility testing was performed using standard microdilution method with minor modifications [25,26]. Briefly, microorganism suspension concentrations from bacterial and yeast stock cultures were adjusted to McFarland 0.5 ($5x10^5$ CFU/mL). Stock solutions of the extract were prepared in dimethyl sulfoxide (DMSO) at 1000 µg/mL. First, 100 µL of Mueller-Hinton broth (MHB) (Merck, Germany) for bacteria and 100 µL of Sabouraud dextrose broth (SDB) (Merck, Germany) for yeast were dispensed into each well of a 96-well microplate. One hundred µL of the stock solution of the extract was added to the first wells, followed by a two-fold dilution. Then, 5 µL of the microorganism (bacteria or yeast) suspension was added to each well. In addition, a medium control well (wells without the addition of the microorganism suspension) and a microbial growth control well (wells without the tested extract) were created. Microplates were incubated for 24 h (37°C for bacteria and 28°C for yeast). The minimum inhibitory concentration (MIC), the lowest concentration at which the tested extract inhibited the growth of the microorganism, was determined via both visual inspection and using a microplate reader (BioTek Inc., USA) at a wavelength of 630 nm. Ampicillin (Sigma, USA) and fluconazole (Sigma, USA) were used as reference drugs. All experiments were repeated 2 times.

2.4. Evaluation of biofilm formation

The biofilm capacity of *P. aeruginosa* was determined to be examined in antibiofilm experiments using the crystal violet (CV) staining method [27]. First, 100 µL of MHB was transferred to 96-well microplate wells and 10 µL of microorganism cell suspensions adjusted to $5x10^5$ CFU/mL were added. To determine biofilm production, the microplate was incubated at 37°C for 24 hours, and after incubation, the cell suspensions were gently aspirated. Then the microplate was rinsed 3 times with sterile PBS to remove non-adherent cells. In the second step, 150 µL of methanol was added to the wells of the microplate and left for 15 min, and the biofilms were fixed. At the end of the period, the methanol in the wells was aspirated and the microplate wells were air-dried. In the third step, 150 µL of 0.5% CV solution was added to the microplate wells and incubated for 15 min. The CV solution in the microplate wells was aspirated, washed with PBS, and air-dried. In the last step, 150 µL of 95% ethanol was added to the wells of the microplate and retained for 15 min. The biofilm formation was determined by measuring the absorbance at optical density (OD) at 550 nm using a microtiter plate spectrophotometer (BioTek Inc., USA). The OD values of the wells without microorganism inoculum were used as the negative control. All tests were performed in duplicate. The biofilm production capacity of the isolate was then determined [28]. Because it is one of the strains with the best biofilm production capacity, biofilm tests were performed on the biofilm of *P. aeruginosa*.

2.5. Biofilm prevention and eradication assays

The effect of the extract both on preformed biofilm and its biofilm inhibition potential was determined with minor modifications in the CV staining experiment [29]. For the biofilm prevention assay, 100 µL of MHB was dispensed into the microplate wells and serial dilutions were made at 0.5x and 0.25x MIC concentrations of the extract. The microorganism suspension adjusted to McFarland 0.5 ($5x10^5$ CFU/mL) was seeded into 96-well microplates and then incubated at 37°C for 24 hours. Next, the potential of the tested extract to inhibit biofilm formation was determined by following the CV staining assay steps as described under the "Evaluation of biofilm formation" section [27]. For biofilm eradication test, 100 µL of MHB was dispensed into 96 microplate wells and 5 µL of McFarland 0.5 adjusted microorganism suspension was added to each well and incubated at 37°C for 24 hours. After incubation, the supernatants were gently aspirated and 100 µL of the 0.5X, 1X, and 2X MIC concentrations of the extracts were added to each well and incubated again at 37°C for 24 hours. The potential of the extract to inhibit preformed biofilm was then determined by following the CV staining test steps mentioned in the "Evaluation of biofilm formation" section. PBS was used as a negative control in both antibiofilm experiments. Both the effect of the extract on preformed biofilm as well as its potential to inhibit biofilm formation was evaluated by measuring the OD of the wells at 550 nm using a microplate reader (BioTek Inc., USA). The "Minimum Biofilm Inhibition Concentration ($MBIC_{50}$)", the lowest extract concentration at which biofilm formation is inhibited by at least 50%, and the "Minimum Biofilm Reduction Concentration ($MBRC_{50}$)", the lowest extract concentration required to destroy at least 50% of the preformed biofilm, were determined.

3. Results and Discussion

In the present study antibacterial, antifungal, and antibiofilm activities of the ethanol extract obtained from mericarps of *S. microstegia* were investigated. The yield of the extract was determined as 31.25% (w/w).

3.1. Antimicrobial activity

In this study, the antimicrobial activity of the extract was tested against seven bacterial and three fungal strains. It was determined that the extract inhibited the growth of bacteria and yeasts tested in the study at concentrations ranging from 62.5 to 250 µg/mL (Table 1). The extract showed moderate and low antimicrobial activity when compared with the reference antimicrobials (fluconazole for yeast, ampicillin for bacteria). In general, it was observed that there was no significant difference for the antimicrobial activity of the extract on the tested organisms. The antimicrobial activity of the extract was significantly greater especially against *K. pneumoniae* and *E. faecalis* at a lower MIC value compared to other bacteria. In addition, it was examined that the antifungal activity of the extract against *C. glabrata* and *C. parapsilosis* was better than those of *C. albicans*.

Table 1. Antifungal and antibacterial properties of ethanolic extract of *S. microstegia* mericarps (µg/mL)

Extract/ Reference antimicrobials	*K. pneumoniae ATCC 100031*	*B. subtilis ATCC 6633*	*P. aeruginosa* ATCC 27853	*S. aureus* ATCC 29213	*E. coli* ATCC 35150	*E. faecalis ATCC 29212*	*A. baumannii ATCC 02026*	*C. albicans ATCC 90028*	*C. glabrata* ATCC 15126	*C. parapsilosis ATCC 90018*
S. microstegia	62,5	125	125	250	125	62,5	125	125	62,5	62,5
Ampicillin	*	*	31,25	*	3,90	*	31,25	-	-	-
Fluconazole	-	-	-	-	-	-	-	*	8	*

-Not tested;* Effective at all tested concentrations

The antimicrobial potential of different *Salvia* species on pathogen microorganisms have been studied previously [15,22-24,30-32]. Aerial parts, root, leaf, stalk, and flower of *S. microstegia* were investigated for their antimicrobial activities against some bacteria and yeast using the disc diffusion method and results indicated that the tested extracts have antimicrobial potential against tested microorganisms except *E. coli, C. albicans,* and *Saccharomyces cerevisiae* [23]. Plant seed fatty acid extract, which was prepared from *n*-hexane, was investigated in the study of Kursat et al. for its antimicrobial activity against *S. aureus* COWAN 1, *B. megaterium* DSM 32, *K. pneumoniae* FMC 66032, *E. coli* ATCC 25922, *C. albicans* FMC 17, *C. glabrata* ATCC 66032, *Trichophyton* sp., and *Epidermophyton* sp. by well agar method and their results showed no antimicrobial activity against the tested organisms except for *S. aureus* and *C. albicans* [22]. In the current study, the ethanol extract of *S. microstegia* mericarps showed various levels of antimicrobial activity against all tested bacteria and yeasts.

3.2. Biofilm assays

The biofilm inhibition test showed that the extract inhibited biofilm formation by approximately 40% and 32% at the concentrations of 0.5x and 0.25X MIC, respectively (Figure 1A). These

data show that the extract reduces biofilm formation at the tested concentrations, but cannot inhibit it up to 50%. Therefore, the studied extract does not show potent $MBIC_{50}$ values for the concentrations tested.

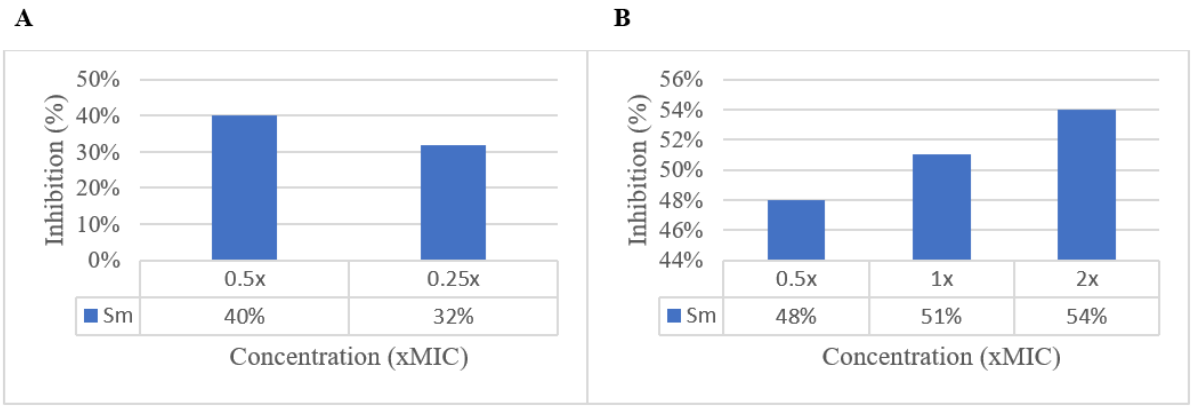

Figure 1. (A) Inhibition of *P. aeruginosa* biofilm formation by *S. microstegia* mericarps in sub-MICs (0.5X and 0.25X) (Sm: *S. microstegia*). **(B)** Preformed *P. aeruginosa* biofilm inhibition of *S. microstegia* mericarps at 0.5X, 1X, and 2X concentrations (Sm: *S.microstegia*).

The approximate percentages of inhibition of biofilm formation by the extract at 0.5X, 1X, and 2X MIC concentrations against *P. aeruginosa* biofilm for 24 hours are shown in Figure 1B. According to these data, the extract was able to reduce the preformed biofilm formation by about 50% at the MIC value (1X). Therefore, the $MBRC_{50}$ value of the extract was 125 µg/mL. In addition, another important result was that 0.5x MIC (62.5 µg/mL) was able to reduce preformed biofilm at a value (48%) which was close to 50% (Figure 1B). In general, the potential of the extract to inhibit preformed biofilm formation was higher than inhibiting biofilm formation.

P. aeruginosa used for biofilm inhibition test is an opportunistic human pathogen that can cause both acute and chronic infections [33]. It can produce a number of virulence factors with its Quorum sensing (QS) regulatory system, which is very important for the formation of biofilm in highly coordinated group behavior and cell-to-cell signaling. Therefore, this species is able to develop antibiotic resistance which could be difficult to eradicate [34].

In this study, the inhibition rate of the preformed biofilm of *P. aeruginosa* was higher than its inhibition of biofilm formation. The obtained data gains importance, especially when considering infections due to *P. aeruginosa* biofilm on biomaterials such as contact lenses, intravenous catheters, implants, and heart valves, and in the lungs of cystic fibrosis patients [34,35]. It is also noteworthy that the extract included in this study inhibited the preformed biofilm of *P. aeruginosa* by nearly 50% in the sub-MIC (0.5X). The studies in the literature show that a higher drug concentrations may be required for the eradication of biofilm producing bacterial cells [10]. However, it is hard to administrate high drug concentrations *in-vivo* due to possible toxicity and side effects. Although the 0.5X MIC concentration (62.5 µg/mL) of the studied extract is not as low as reference drugs, it showed antibiofilm activity.

To the best of our knowledge, the antibiofilm activity of *S.microstegia* was demonstrated for the first time in this study. Thus, it provides preliminary data for the investigation of other species belonging to the genus *Salvia*. In future studies, investigating the possible synergistic effects of this extract with other extracts and researching its antimicrobial and antibiofilm activity may be important in terms of evaluating *S. microstegia* mericarp extract as a potential antimicrobial active ingredient.

4. Conclusion

In recent years, research on the antimicrobial activity of plant-based materials has increased due to their ease of accessibility, low cost, and the lack of resistance development. In conclusion, our findings show that, together with the identification of individual compounds that cause the desired biological effect and the elucidation of their mechanism of action, it will bring a new perspective to evaluate other species of *Salvia* genus as antimicrobial agents in infectious diseases.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Design of the study: ZÖ, SGK; Collection of plant samples: SGK, AK; Performing the experiments: ZÖ, SGK; Evaluation of the results: ZÖ, SGK, AK; Writing of the article: ZÖ, SGK; Review and correction: All authors

References

[1] Morrison, L., Zembower, T.R., 2020. "Antimicrobial Resistance". *Gastrointest Endosc Clin N Am*. 30(4):619-635.

[2] Vasudevan, R., 2014. "Biofilms: microbial cities of scientific significance," *Journal of Microbiology & Experimentation,* 1(3), 1–16.

[3] Del Pozo, J. L. 2018., "Biofilm-related disease", *Expert Rev Anti Infect Ther*. 16(1), 51-65.

[4] Ibberson, C. B., 2020. "Whiteley, M. The social life of microbes in chronic infection", *Curr. Opin. Microbiol.* 53,44–50.

[5] Grant, S. S. and Hung, D. T. 2013., "Persistent bacterial infections,antibiotic tolerance, and the oxidative stress response," *Virulence,* 4(4),273–283.

[6] Bjarnsholt, T. 2013., "The role of bacterial biofilms in chronic infections". *APMIS Suppl*. 136,1-51.

[7] Melandera, R. J., Basaka, A. K., Melandera, C., 2020. "Natural Products as Inspiration for the Development of Bacterial Antibiofilm Agents". *Nat Prod Rep*. 37(11),1454–1477.

[8] Alekshun, M. N. and Levy, S. B., 2007. "Molecular mechanisms of antibacterial multidrug resistance," *Cell,* 128(6),1037– 1050.

[9] Macia, M. D., Rojo-Molinero, E., Oliver, A., 2014. "Antimicrobial susceptibility testing in biofilm-growing bacteria". Clin Microbiol Infect. 20(10),981-90.

[10] Hengzhuang, W., Wu, H., Ciofu, O., Song, Z., Hoiby, N., 2012. "In vivo pharmacokinetics/pharmacodynamics of colistin and imipenem in Pseudomonas aeruginosa biofilm infection". *Antimicrobial Agents Chemotherapy* 56,2683-90.

[11] Kartal, M., 2007. "Intellectual property protection in the natural product drug discovery, traditional herbal medicine and herbal medicinal products". *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 21(2), 113-119.

[12] Nascimento, G. G., Locatelli, J., Freitas, P. C., & Silva, G. L., 2000. "Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria". *Brazilian Journal of Microbiology*, 31(4), 247-256.

[13] Drew, B. T., González-Gallegos, J. G., Xiang, C. L., Kriebel, R., Drummond, C. P. & Walker, J., 2017. "*Salvia* united: the greatest good for the greatest number". *Taxon* 66,133-145.

[14] Hatipoglu, S. D., Zorlu, N., Dirmenci, T., Goren, A. C., Ozturk, T. & Topcu, G., 2016. "Determination of volatile organic compounds in fourty five *Salvia* species by thermal desorption-GC-MS technique". *Records of Natural Products* 10(6): 659-700.

[15] Güzel, S., Özay, Y., Ülger, M., Kahraman, A., Aslan, G., 2018. "Evaluation of antimicrobial activity of three endemic *Salvia* species growing wild in Turkey". *Hacettepe University Journal of the Faculty of Pharmacy*, 38(1), 4-10.

[16] Pitarokili D, Tzakou O & Loukis A. 2006. "Essential oil composition of *Salvia verticillata, S. verbenaca, S. glutinosa* and *S. candidissima* growing wild in Greece". *Flavour and Fragrance Journal* 21,670-673.

[17] Bakoğlu, A., Kılıç, O. & Kökten, K., 2016. "Fatty acid composition of the leaves of some *Salvia* taxa from Turkey". *Chemistry of Natural Compounds* 52(4), 676-678.

[18] Topçu, G., Yücer, R. & Şenol, H., 2017. Bioactive constituents of Anatolian *Salvia* species. In: GEORGIEV V & Pavlov A. (ed), , 2017. *Salvia* Biotechnology. pp 31-132, Springer International Publishing AG. Springer, Cham.

[19] Adımcılar, V., Kalaycıoğlu, Z., Aydoğdu, N., Dirmenci, T., Kahraman, A. & Erim, F. B., 2019. "Rosmarinic and carnosic acid contents and correlated antioxidant andantidiabetic activities of 14 *Salvia* species from Anatolia". *Journal of Pharmaceutical and Biomedical Analysis* 175, 112763-68.

[20] Bayar, Y. & Genç, N., 2018. "Determination of the chemical components, antioxidant and antifungal activities of essential oil and plant extract of *Salvia candidissima* Vahl." *Mediterranean Agricultural Sciences* 31(2), 93-99.

[21] Yapar, Y., Behçet, L., 2018 "The flora of Hiro Plateau (Adaklı-Bingöl/ Turkey) and its surroundings". *Biological Diversity and Conservation* 11(3), 126-140.

[22] Kürşat, M., Erecevit, P., Sarı, A., Emre, İ., Kırbağ, S. & Civelek, Ş., 2012. "The antimicrobial activities of seed fatty acid extracts from some *Salvia* L. Species". *Turkish Journal of Science & Technology* 7(1),31-36.

[23] Erbil, N. and Dıgrak, M. 2015., "Total phenolic and flavonoid contents, antimicrobial and antioxidant Properties of *Salvia verticillata* L. var. *amasiaca* and *Salvia microstegia* Boiss & Bal from Turkish Flora". *Journal of Microbiology and Antimicrobial Agents* 1(1), 23–29.

[24] Güzel, S., Ülger, M., Kahraman, A. 2020., "Chemical composition and some biological activities of *Salvia longipedicellata* Hedge mericarps". *Chemistry of Natural Compounds*, 56(5), 788-792.

[25] Woods, G. L., Washington, J. A., 1995. Antibacterial susceptibility tests: dilution and disk diffusion methods. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, (eds). Manual of clinical microbiology. 6th ed, Washington, DC: American Society for Microbiology. 1327–41.

[26] Jorgensen, J. H., Ferraro. M. J. 1998. "Antimicrobial susceptibility testing: general principles and contemporary practice". *Clin Infect Dis* 26,973–80.

[27] O'Toole, G. A., 2011. "Microtiter dish biofilm formation assay". *J Vis Exp* 47,2437.

[28] Tekintaş, Y., Temel, A., Ateş, A., Eraç, B., Metin, D.Y., Hilmioğlu Polat, S,, Hoşgör Limoncu, M. 2020. "Antifungal and antibiofilm activities of selective serotonin reuptake inhibitors alone and in combination with Fluconazole". *Turk J Pharm Sci*. 17(6),667-672.

[29] Zhong, H., Xie, Z., Wei, H., Zhang, S., Song, Y., Wang, M., and Zhang, Y., 2019. "Antibacterial and antibiofilm activity of temporin-GHc and temporin-GHd against cariogenic bacteria, *Streptococcus mutans*". *Front Microbiol.* 10, 2854.

[30] Güzel, S., Kahraman, A.,; Ülger, M., Özay, Y., Bozgeyik, I., Sarıkaya, Ö., 2019. "Morphology, myxocarpy, mineral content, and *in vitro* antimicrobial and antiproliferative activities of mericarps of the vulnerable Turkish endemic *Salvia pilifera"*. *Journal of Research in Pharmacy*, 23,729-739.

[31] Güzel, S., Özay, Y., Kumaş, M., Uzun, C., Özkorkmaz, E., Yıldırım, Z., Ülger, M., Güler, G., Çelik, A., Çamlıca, Y., Kahraman, A., 2019. "Wound healing properties, antimicrobial and antioxidant activities of *Salvia kronenburgii* Rech. f. and *Salvia euphratica* Montbret, Aucher

& Rech. f. var. *euphratica* on excision and incision wound models in diabetic rats". *Bıomedıcıne & Pharmacotherapy*, 11,1260-1276.

[32] Güzel, S., Ülger, M., Kahraman, A., 2021. "Phytochemical analysis, antioxidant and antimicrobial activities of *Salvia virgata* mericarps". *Botanica Serbica*, 45(2), 223-231.

[33] Thi, M. T. T., Wibowo, D., Rehm, B. H. A., 2020. "Pseudomonas aeruginosa biofilms". *Int J Mol Sci* 21,8671.

[34] Ben Haj Khalifa, A., Moissenet, D., Vu Thien, H., Khedher, M., 2011. "Virulence factors in *Pseudomonas aeruginosa*: mechanisms and modes of regulation". *Annales de biologie clinique* 69(4),393–403.

[35] Lindsay, D., von Holy, A., 2006. "Bacterial biofilms within the clinical setting: what healthcare professionals should know". *J Hosp Infect*. 64(4),313-25.