



CHEMICAL COMPOSITION AND BIOACTIVITIES OF ESSENTIAL OIL FROM AN ENDEMIC *SALVIA ABSCONDITIFLORA* GREUTER & BURDET

ENDEMİK SALVIA ABSCONDITIFLORA GREUTER & BURDET UÇUCU YAĞININ KİMYASAL İÇERİĞİ VE BİYOAKTİVİTELERİ

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ABSTRACT

Objective: The study aimed to determine the chemical composition and antibacterial, antibiofilm, and anti-quorum sensing activities of the essential oil of *Salvia absconditiflora* Greuter & Burdet (an endemic species) growing wildly in Türkiye.

Material and Method: The essential oil from the aerial parts of the plant was obtained by hydro-distillation (0.4%) and analyzed by GC-FID and GC-MS. In addition, the broth microdilution method was used to determine antibacterial activity. The crystal violet assay was performed for antibiofilm activity, and the reporter bacteria *Chromobacterium violaceum* ATCC 12472 was used in the anti-quorum sensing activity test.

Result and Discussion: The major components of the essential oil were identified as 1,8-cineole (32.2%), camphor (13.6%), α -pinene (7.6%), camphene (5.5%), and viridiflorol (5.1%). The essential oil showed the best antibacterial activity against Gram-positive test bacteria, with a minimum inhibitory concentration (MIC) of 0.0078 (v/v) against *Staphylococcus aureus* strains. The percentage biofilm inhibition value of the essential oil was determined as 84.4%. The inhibition of violacein production by the essential oil in *Chromobacterium violaceum* ATCC 12472 indicated the possibility of anti-quorum sensing activity. The results of this study show that the essential oil of *S. absconditiflora* could be a promising alternative in fighting bacterial infections.

Keywords: Antibacterial activity, antibiofilm activity, anti-quorum sensing activity, essential oil, Lamiaceae, *Salvia absconditiflora*

ÖZ

Amaç: Bu çalışma, Türkiye'de doğal olarak yetişen *Salvia absconditiflora* Greuter & Burdet (endemik) uçucu yağının kimyasal bileşimini ve antibakteriyel, antibiyofilm ve anti-quorum sensing aktivitelerini belirlemeyi amaçlamıştır.

Gereç ve Yöntem: Bitkinin toprak üstü kısımlarından su distilasyonu yöntemi ile elde edilen uçucu yağın verimi (%0.4) belirlenmiş, GC-FID ve GC-MS cihazları ile kimyasal içeriği tayin edilmiştir. Ayrıca *S. absconditiflora*'dan elde edilen uçucu yağın antibakteriyel aktivitesi mikrodilüsyon yöntemi ile belirlenmiştir. Antibiyofilm aktivite için kristal viyole yöntemi ve anti-quorum sensing

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aktivite için raportör bakteri *Chromobacterium violaceum* ATCC 12472 kullanılmıştır.

Sonuç ve Tartışma: Uçucu yağın ana bileşenleri 1,8-sineol (%32.2), kafur (%13.6), α -pinen (%7.6), kamfen (%5.5) ve viridiflorol (%5.1) olarak belirlenmiştir. Uçucu yağ, Gram-pozitif bakterilerine karşı en iyi antibakteriyel aktiviteyi, *Staphylococcus aureus* suşlarına karşı 0,0078 (h/h) minimum inhibitör konsantrasyonu (MIC) ile göstermiştir. Ayrıca uçucu yağın biyofilm inhibisyon değeri %84.4 olarak belirlenmiştir. *Chromobacterium violaceum* ATCC 12472 suşunda viyolasin üretiminin engellenmesi de, olası anti-quorum sensing aktivitesinin varlığını göstermiştir. Yapılan çalışmaların sonucunda, *S. absconditiflora* uçucu yağının bakteriyel enfeksiyonlarla mücadelede umut verici bir alternatif olabileceği belirlenmiştir.

Anahtar Kelimeler: Antibakteriyel aktivite, antibiyofilm aktivite, anti-quorum sensing aktivite, Lamiaceae, *Salvia absconditiflora*, uçucu yağ

INTRODUCTION

Plants and their secondary metabolites have an important place in traditional medicine. People use herbs to improve their health. Herbs will continue to play a significant role as a tool in health related to current research and investments [1]. Plant bioactive compounds have been an attractive target due to their biological effects and potential for biotechnological use [2]. Volatile oils are produced in various plant organs and have numberless healing effects in human diseases [3]. The Lamiaceae (Labiatae) family, which includes about 7200 species in 237 genera, mainly spreads in the Mediterranean and Central Asia [4]. A reputable aromatic and medicinal plant, *Salvia* L. (sage), a relatively large genus in Lamiaceae, comprising over 900 species in the world [5], is represented naturally by 89 species (with 50% endemism) in the "Flora of Turkey." Anatolia is an important gene center in Asia [6-8]. The name *Salvia* is derived from "salvare," meaning "to heal" and "to save" in Latin [9]. Most *Salvia* species are native to the Mediterranean region and are traditionally used for bronchitis, cough, asthma, digestive and circulatory disorders, excessive sweating, angina, inflammation, memory problems, and depression [10]. These species are rich sources of polyphenols, diterpenes, and triterpenes [9-12] and are also rich in essential oils. The *Salvia* species mainly exhibits antimicrobial, antioxidant, anti-inflammatory, antidiabetic, antiviral, hepatoprotective, anticancer, and antidepressant effects [9,13-15].

Salvia absconditiflora Greuter & Burdet (Syn: *Salvia cryptantha* Montbret & Aucher ex Benth), which has been used in folk medicine for many years, is endemic to Türkiye. *S. absconditiflora* called "tapir" in Turkish, is a perennial herb that grows at an altitude of 700 to 2500 m on rocky lands, dry places, calcareous hills, and fallow lands [6]. According to regional knowledge, *S. absconditiflora*, is a wild plant in Central Anatolia, and its flowers in dry form are used as herbal tea [16]. Studies have mostly been done on this species regarding essential oil composition collected from different localities [17,18]. In a study, *S. absconditiflora* was collected from eight different provinces of Türkiye, and its phytogeographic effects on essential oil composition were examined. The main components of all species were demonstrated as α -pinene, camphene, 1,8-cineole, camphor, and borneol [18]. In a different study investigating the antimicrobial effects of essential oil of six species growing in Türkiye, including *S. absconditiflora*, the activity of this species was observed at low or medium levels [19]. Additionally, the antifungal and bioherbicidal properties of *S. absconditiflora* essential oil have been reported to use as a natural fungicide and herbicide due to its potent activity [20].

Antibiotic resistance is one of the most critical health concerns worldwide, as new strains of resistant bacteria have been reported from different countries. Infections are presently a significant cause of these bacteria for morbidity and mortality. All these consequences bring us to find urgently new compounds with antibacterial effects to treat these diseases that emerged from resistant bacteria. The difficulties in discovering new antibacterials and the need for long research have led researchers to discover new molecules that can inhibit other mechanisms involved in pathogenicity [21,22]. One of those mechanisms, Quorum Sensing (QS), effectively synthesizes virulence factors contributing to pathogenicity and biofilm formation. Scientists have recently focused on antibiofilm and anti-quorum sensing molecules as alternative compounds to treat bacterial infections. Based on the current research, anti-quorum sensing and antibiofilm compounds, especially by scanning natural resources, would effectively control the resistance problem [23-26]. Since the beginning of human history, plants and essential oils have been used for different purposes. One of the most important effects of many essential

oils is their antimicrobial potential. They seem to be a potential alternative to synthetic compounds, mainly due to the increasingly developed resistance of pathogenic microorganisms [27].

Therefore, in this current study, the essential oil composition of *Salvia absconditiflora* was investigated for the first time collected from the indicated locality, as well as a comprehensive evaluation of its antibacterial, antibiofilm, and anti-quorum sensing activity.

MATERIAL AND METHOD

Plant Material

An endemic species, *Salvia absconditiflora* Greuter & Burdet (Syn: *Salvia cryptantha* Montbret & Aucher ex Benth) from the Lamiaceae family was collected from the Ankara-Eskişehir Road in Türkiye on 12/06/2021. Taxonomic identification of the plant material was confirmed by Prof. Dr. Hayri Duman from the Department of Biology, Gazi University. The voucher specimen was deposited at the Herbarium of Ankara University (AEF) with the registered number AEF 28898.

Essential Oil Distillation

The air-dried aerial parts (with flowers) of the plant in the flowering period were subjected to hydro-distillation for 3-4 hrs. using a Clevenger-type apparatus. The essential oil was dehydrated on anhydrous sodium sulfate, filtrated, and kept at +4°C until tested and analyzed. The oil yield was 0.4% v/w (200 g of the plant gave 0.8 ml, 455 mg oil).

Gas Chromatography (GC) Analysis

An Agilent 6890N GC system was used for GC analysis. The FID detector temperature was adjusted to 300°C, and simultaneous automatic injection was performed to replicate the same column, applying the same process conditions to achieve the same elution order as by GC-MS. The relative percentage amounts of the separated components were determined with the help of FID chromatograms, and the analysis results are given in Table 1. The essential oil components were identified by comparing their relative retention times with the original samples or their relative retention indices (RRI) with a range of n-alkanes. Commercial libraries are known as Wiley GC/MS Library, and MassFinder Software 4.0 [28,29] was used to identify components. In addition, a computer comparison with the in-house "Baser Essential Oil Components Library" consisting of original compounds and components of known oils was also used to determine the essential oil composition.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis was carried out with an Agilent 5975 GC-MSD system. Innovax FSC column (60 m x 0.25 mm, 0.25 µm 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, kept constant at 220°C for 10 min, and then programmed to 240°C at a rate of 1°C/min. The split ratio was kept to 40:1. The injector temperature was set at 250°C. Mass spectra were recorded at 70 eV. The mass range was from m/z 35 to 450.

Antibacterial Activity

The essential oil's MIC (Minimum Inhibitory Concentration) values were determined by the broth microdilution method. *Staphylococcus aureus* ATCC 25923, methicillin-resistant *S. aureus* ATCC 43300 (MRSA), *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883 were used as test bacteria [26,30]. Serial two-fold dilutions of essential oil (0.25 to 0.002 v/v) were prepared in Mueller Hinton Broth (Difco, Difco Laboratories, Detroit, MI, USA) supplemented with Tween 80 (Merck, Germany) to adjust a final concentration of 0.5% (v/v). The final test concentration of the bacteria was adjusted to 5×10^5 CFU/ml. After the incubation at 35°C for 18-24 hrs., the last well that completely inhibited visual bacterial growth was noted as the MIC value. A set of wells containing only inoculated broth supplemented with Tween 80 was used as the negative control.

Antibiofilm Activity

Before performing the antibiofilm activity test, the MIC value of the essential oil against *Pseudomonas aeruginosa* PAO1 was determined. Antibacterial activity was not observed. The antibiofilm activity test was performed by *in vitro* microplate-based biofilm model against *P. aeruginosa* PAO1 using the crystal violet assay [26,31-33].

For biofilm formation, *P. aeruginosa* PAO1 was incubated for 24 hrs at 37°C in Brain Heart Infusion (BHI) Broth. The final inoculum suspension containing *P. aeruginosa* (~10⁶ CFU/ml) was prepared in BHI enriched with 2% sucrose. 100 µl of the inoculum suspensions were added to 96-well microtiter plates for all test conditions. The plates were incubated at 37°C for 72 hrs. to form mature biofilm.

After forming the mature biofilm layers, the medium was aspirated, and non-adhered cells were removed by washing the wells with sterile phosphate-buffered saline (PBS, pH 7.2). 100 µl of essential oil was transferred into each well containing mature *P. aeruginosa* biofilms. The plates were incubated at 37°C for 24 hrs. After incubation, the content of the wells was poured off, and the wells were washed with PBS. The plates were then dried at room temperature for 1 hr. 100 µl of 0.5% crystal violet solution was added to each well for staining the biofilm cells. After 30 min, the wells were washed three times with PBS. Then, acetone-alcohol (30:70 v/v) solution was added to the wells to dissolve the bound dye within the biofilm matrix. BHI Broth enriched with 2% sucrose was used as a control. The optical density of the dissolved crystal violet dye was measured by a microplate reader (Thermo Scientific Multiskan GO Microplate Spectrophotometer, Vantaa, Finland) at 620 nm (OD 620 nm). The percentage biofilm inhibition values were calculated according to the following formula:

$$\% \text{ Biofilm inhibition} = [(OD (\text{Growth control}) - OD (\text{Sample})) / OD (\text{Growth control})] \times 100$$

Antiquorum Sensing Activity

The disc diffusion method was used to perform the anti-quorum sensing activity test. *Chromobacterium violaceum* ATCC 12472 was used as the reporter bacteria [26,34,35]. The density of the overnight bacterial culture was adjusted to 1.5x10⁸ CFU/ml. Then, the bacterial suspension was inoculated on Luria Bertani Agar, and a sterile blank disc (6 mm diameter; Bioanalyse®, Ankara, Türkiye) impregnated with twenty microliters of the essential oil (0.15 mg/µl) was placed on the medium. Luria Bertani Broth was used as the negative control. After incubation at 30°C for 24 hrs, the plates were observed for a zone of violacein inhibition. The formation of an inhibition zone around the disc was noted as the potential anti-quorum sensing activity.

RESULT AND DISCUSSION

The present study reported the essential oil composition of *Salvia absconditiflora* with antibacterial, antibiofilm, and anti-quorum sensing activities. Traditionally, people have used *Salvia* species for various ailments, which may be the main components responsible for their biological properties.

Ordinarily, in *Salvia* species, essential oils of all aerial parts found in the glandular trichomes were an average concentration of 1.3-3.6% by dry weight, almost all. In comparison, the essential oil rate is maximum in leaves, low in flowers, and lowest in branches. In previous studies, both aerial parts and flower essential oils of *S. absconditiflora*; have the major components as 1,8-cineole (30.38 and 36.28%), valencene (24.34 and 26.53%), and camphor (12.29 and 14.72%) [36]. In addition, valencene (31.80%) was also determined as the leading compound from the aerial parts of this plant in native and field-grown species [37]. Moreover, the seed oils of several *Salvia* species, including *S. absconditiflora* were investigated and the γ -tocopherol was abundant component in most of the seed oils [38]. The significant components in *S. absconditiflora* from Southern Turkish forms were determined as camphor (25.6%) and 1,8-cineole (20.3%), which could be used as flavor and fragrance agents in some products [39].

In the current study, the essential oil concentration was 0.4% in *S. absconditiflora*. The composition of hydrodistilled essential oil from aerial parts almost consisted of flowers of *S.*

absconditiflora was determined by Gas Chromatography-Flame Ionization Detection (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS) simultaneously. The study of the volatiles of *S. absconditiflora* revealed 48 different components, representing 93.5% of its chemical composition. The main components were 1,8-cineole (32.2%), camphor (13.6%), α -pinene (7.6%), camphene (5.5%), and viridiflorol (5.1%) in the essential oil of *S. absconditiflora* (Table 1).

Table 1. The composition of essential oil from *Salvia absconditifolia* Greuter & Burdet

No	RRI	Components	%	IM
1	1014	Tricyclene	0.2	RRI, MS
2	1032	α-Pinene	7.6	RRI, MS
3	1076	Camphene	5.5	RRI, MS
4	1118	β -Pinene	3.8	RRI, MS
5	1132	Sabinene	0.3	RRI, MS
6	1174	Myrcene	0.8	RRI, MS
7	1213	1,8-Cineole	32.2	RRI, MS
8	1246	(Z)- β -Ocimene	0.3	MS
9	1255	γ -Terpinene	0.5	RRI, MS
10	1266	(E)- β -Ocimene	0.1	MS
11	1280	p-Cymene	0.3	RRI, MS
12	1290	Terpinolene	0.1	RRI, MS
13	1452	1-Octen-3-ol	0.1	MS
14	1474	trans-Sabinene hydrate	0.4	MS
15	1466	α -Cubebene	2.0	MS
16	1493	α -Ylangene	tr	MS
17	1532	Camphor	13.6	RRI, MS
18	1535	β -Bourbonene	0.7	MS
19	1590	Bornyl acetate	0.5	RRI, MS
20	1612	β -Caryophyllene	2.5	RRI, MS
21	1611	Terpinen-4-ol	1.0	RRI, MS
22	1719	Borneol	2.1	RRI, MS
23	1704	γ -Muurolene	4.3	MS
24	1740	α -Muurolene	0.6	MS
25	1742	β -Selinene	0.3	MS
26	1744	α -Selinene	0.9	MS
27	1776	γ -Cadinene	2.3	MS
28	1796	Selina-3,7(11)-diene	0.2	MS
29	1799	Cadina-1,4-diene	0.2	MS
30	1804	Mrytenol	0.3	MS
31	1807	α -Cadinene	0.1	MS
32	1849	Calamelene	0.6	MS
33	1900	epi-Cubebol	0.2	MS
34	1941	α -Calacorene	0.2	MS
35	1953	Palustrol	0.4	MS
36	1984	γ -Calacorene	0.1	MS
37	2008	Caryophyllene oxide	0.8	RRI, MS
38	2057	Ledol	0.3	MS
39	2088	1-epi-Cubenol	0.1	MS
40	2104	Viridiflorol	5.1	MS
41	2145	Valeranone	0.7	MS
42	2257	β -Eudesmol	0.7	MS
43	2324	Caryophylladienol II	0.1	MS
44	2392	Caryophyllenol II	0.1	MS
45	2500	Pentacosane	0.1	RRI, MS
46	2622	Phytol	0.1	MS

Table 1 (continue). The composition of essential oil from *Salvia absconditiflora* Greuter & Burdet

No	RRI	Components	%	IM
47	2600	Hexacosane	0.1	RRI, MS
48	2670	Tetradecanoic acid	tr	RRI, MS
Total			93.5	

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

IM: Identification method, RRI, identification based on the relative retention times of genuine compounds on the HP Innowax column; MS, identified based on computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

Monoterpene hydrocarbons 19.5 %, Oxygenetaed monoterpenes 50.1 %

Sesquiterpene hydrocarbons 15.0 %, Oxygenetaed sesquiterpenes 8.5 %

Fatty acid+esters (tr), Diterpenes 0.1 %, Others 0.3 %

Herbs and essential oils have many pharmacological properties that are used in medicine because of their health properties. Essential oils have been used in many fields for significant antimicrobial effects [27]. Many recent articles have reported the antimicrobial properties of extracts, essential oils, resins, and various plant phytochemicals. However, detailed studies on plant-derived antimicrobial agents used in practical applications to improve human health are still incomplete [40]. In a previous study, the methanolic extract of *S. absconditiflora* showed inhibitory activity against three strains of HRoV (EC₅₀ values ranged from 5.8 µg/ml to 25.5 µg/ml) in a dose-related manner. Moreover, it was inactive or hardly active against other RNA viruses called human rhinovirus and respiratory syncytial virus [41]. The ethanolic extract *S. absconditiflora* exhibited marked results in wound healing activity in rats [42]. The essential oil of *S. absconditiflora* also inhibits the growth of pathogenic microorganisms as scavenging free radicals [43]. A study also concluded that *S. absconditiflora* (black weed) extract protects against liver damage caused by CCl₄-induced and may be helpful as a hepatoprotective agent to combat the toxic effects caused by CCl₄ and other chemicals [44]. *S. absconditiflora* is also in a formulation called YXFMs (Yixin-Fumai granules), used in Chinese traditional medicine to treat bradyarrhythmia and increase heart rate besides being effective as a remedy for sick sinus syndrome [45]. In another study, the significant component of *S. absconditiflora* oil, 1,8-cineole, is a component of many drugs due to its anti-inflammatory, mucolytic, antiseptic, and antimicrobial properties [46,47].

On the other hand, camphor possesses many biological activities, including antiviral and antimicrobial activities [48]. Essential oils containing 1,8-cineole have been reported to be used as folk remedies for decades. In addition, several studies have revealed that 1,8-cineole has been used as an active component in various diseases [47]. Gum Arabica, obtained from *Acacia* species, is a commercial natural product that contains many active bio components. The extracts of this product inhibited violacein production at MIC and sub-MIC concentrations in *Chromobacterium violaceum* CV12472 and nucleation detection under externally supplied acyl-homoserine lactone. The extracts also exhibited antimicrobial activity (MIC = 0.1562 mg/ml-2.5 mg/ml), especially at MIC and lower MIC concentrations, showed excellent biofilm inhibition against *E coli* [49]. Propolis is one of the trendy traditional medicines due to its antimicrobial activity and antioxidant effects. The extract had a notable activity with an inhibition diameter zone of 18.0±1.0 mm, and its active components were also determined. Samples blocked *P. aeruginosa* PA01 herd motility at the three concentrations (50, 75, and 100 g/ml) in a dose-dependently. This completed study reported that Propolis extract and its components inhibit biofilm formation [50].

In this context, we investigated the antibacterial (Table 2), antibiofilm, and anti-quorum sensing activities of *S. absconditiflora* essential oil. In the present study, 1,8-cineole or eucalyptol is the major component (32.2%) in the essential oil of *S. absconditiflora*. The current analysis of the essential oil of *S. absconditiflora* exhibited marked antibacterial activity against Gram-positive test bacteria with MIC values between 0.0078 and 0.0625 (v/v). For this reason, the notable antimicrobial effect of the *S. absconditiflora* essential oil is attributed to these major components, especially the 1,8-cineole.

Biofilms are defined as intense populations of sessile bacterial cells adhering to the surface and forming a protein, DNA, and exopolysaccharide matrix. The biofilm formation mechanism of this matrix, called extracellular polymeric substance, occurs by providing stability to the cells holding it. It also assisted in the pathogenesis of biofilm-associated infections and resistance by providing nutrients.

Biofilms promote bacterial persistence by resisting host immune responses and antibiotic treatment [51]. This study determined the percentage biofilm inhibition value of tested essential oil to be 84.4%. According to the results, the essential oil has notable antibiofilm activity (Figure 1).

Table 2. Minimum inhibitory concentration (MIC) values of essential oil (v/v) against tested bacteria.

Essential Oil	Gram-positive Test Bacteria			Gram-negative Test Bacteria		
	<i>S. aureus</i> ATCC 25923	<i>S. aureus</i> ATCC 43300 (MRSA)	<i>E. faecalis</i> ATCC 29212	<i>E. coli</i> ATCC 25922	<i>K. pneumoniae</i> ATCC 13883	<i>P. aeruginosa</i> ATCC 27853
EO	0.0078	0.0078	0.0625	0.0625	0.125	-
DMSO (10%)	-	-	-	-	-	-

'-': represents no activity

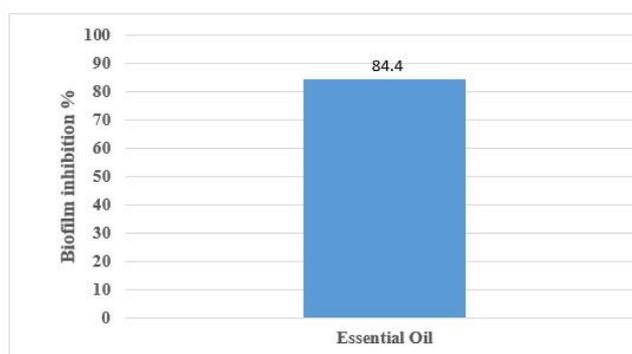


Figure 1. Antibiofilm activity of essential oil of *Salvia absconditiflora*

The global threat of antimicrobial resistance development leads to a constant demand to discover new antimicrobial drugs and antiviral agents. The so-called 'quorum-sensing' is a signaling system that regulates cellular processes such as bacterial cell-to-cell communication [52]. Thus, objection to the bacterial pathogenic potential through quorum-sensing inhibition is welcomed as a new strategy to struggle with microbial resistance [53,54]. The anti-quorum sensing activity inhibited the quorum-sensing-controlled violacein pigment production by the sensor bacteria. The disc produces a transparent inhibition zone representing the potency of quorum-sensing's inhibitory effect. This effect tested on the essential oil is shown in Figure 2.



Figure 2. QS inhibitory activity of essential oil of *Salvia absconditiflora*
SAEO: *Salvia absconditiflora* essential oil, C: Control, LB Broth

For all these reasons, quorum sensing inhibition is new hope in tackling multi-antibiotic-resistant bacteria. Instead of bactericidal or bacteriostatic strategies, inhibition of bacterial nucleotide detection systems is thought to find applications in medicine, agriculture, and food technology [55,56].

The random use of antimicrobial drugs has led to the rise of resistant bacteria, fungi, and viruses. Developing more effective antimicrobial agents with the help of new mechanisms on activities is necessary to overcome the increased resistance of pathogenic microbes. Medicinal and aromatic plants that treat various diseases appear to be the source of secondary metabolites and essential oils. For this reason, studies on the screening of these metabolites of plants in terms of antimicrobial activities are increasing. Because of their effects, essential oils can be considered an alternative to antibiotics. The results support the medical application of these oils to prevent and treat certain infections and diseases and suggest further studies. In conclusion, the essential oil of *Salvia absconditiflora* may represent a new source of antibacterials in the future treatment of bacterial infection-related ailments in the pharmaceutical and food industry.

AUTHOR CONTRIBUTIONS

Concept: A.O.; Design: A.S.C.K., A.O.; Control: A.O.; Sources: A.S.C.K., A.O.; Materials: A.O.; Data Collection and/or Processing: A.S.C.K., S.S.R., M.E., B.D., A.O.; Analysis and/or Interpretation: M.E., B.D.; Literature Review: A.S.C.K., A.O.; Manuscript Writing: M.E., A.O.; Critical Review: A.S.C.K., S.S.R., M.E., B.D., A.O.; Other: -

CONFLICTS OF INTEREST

The authors declare that this article has no real, potential, or perceived conflict of interest.

ETHICS COMMITTEE APPROVAL

The authors declare that ethics committee approval is not required for this study.

REFERENCES

1. Sofowora, A., Ogunbodede, E., Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional, Complementary and Alternative Medicines*, 10(5), 210-229. [\[CrossRef\]](#)
2. Tako, E. (2020). Dietary plant-origin bio-active compounds, intestinal functionality, and microbiome. *Nutrients*, 12(11), 3223. [\[CrossRef\]](#)
3. Dhifi, W., Sana Bellili, S., Jazi, S., Bahloul, N., Wissem Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. *Medicines (Basel)*, 3(4), 25. [\[CrossRef\]](#)
4. Karpinski, T.M. (2020). Essential oils of Lamiaceae family plants as antifungals. *Biomolecules*, 10, 103. [\[CrossRef\]](#)
5. Standley, P., Williams, L. (1973). *Labiatae. Fieldiana Botany*, 24, p.237-317.
6. Davis, P.H. (1982). *Flora of Turkey and the East Aegean Island*. Vol. 7. Edinburgh University Press. p. 433-434.
7. Davis, P.H. (1988). *Flora of Turkey and the East Aegean Island*. Vol.10. Edinburgh University Press. p. 150-151.
8. Güner, A., Özhatay, N., Ekim, T., Baser, K.H.C. (2000). *Flora of Turkey and the East Aegean Island*. Vol. 11, Suppl. Edinburgh University Press. p. 141.
9. Abd Rashed, A., Rathi, D.N.G. (2021). Bioactive components of *Salvia* and their potential antidiabetic properties: A Review. *Molecules*, 26, 3042. [\[CrossRef\]](#)
10. Lopresti, A.L. (2017). *Salvia* (Sage): A Review of its potential cognitive-enhancing and protective effects. *Drugs in R&D*, 17(1), 53-64. [\[CrossRef\]](#)
11. Lu, Y., Foo, L.Y. (2002). Polyphenolics of *Salvia*-A review. *Phytochemistry*, 59(2), 117-40. [\[CrossRef\]](#)
12. Xu, J., Wei, K., Zhang, G., Lei, L., Yang, D., Wang, W., Han, Q., Xia, Y., Bi, Y., Yang, M., Yang, M., Li, M. (2018). Ethnopharmacology, phytochemistry, and pharmacology of Chinese *Salvia* species: A review. *Journal of Ethnopharmacology*, 225, 18-30. [\[CrossRef\]](#)
13. Tosun, A., Khan, S., Kim, Y.S., Calín-Sánchez, Á., Hysenaj, X., Carbonell-Barrachina, A. (2014). Essential

- oil composition and anti-inflammatory activity of *Salvia officinalis* L. (Lamiaceae) in murin macrophages. *Tropical Journal of Pharmaceutical Research*, 13(6), 937-942. [CrossRef]
14. Askari, S.F., Avan, R., Tayarani-Najaran, Z., Sahebkar, A., Eghbali, S. (2021). Iranian *Salvia* species: A phytochemical and pharmacological update. *Phytochemistry*, 183, 112619. [CrossRef]
 15. Onder, A., Izgi, M.N., Cinar, A.S., Zengin, G., Yilmaz, M.A. (2022) The characterization of phenolic compounds via LC-ESI-MS/MS, antioxidant, enzyme inhibitory activities of *Salvia absconditiflora*, *Salvia sclarea*, and *Salvia palaestina*: A comparative analysis. *South African Journal of Botany*, 150, 313-322. [CrossRef]
 16. Bellomaria, B., Arnold, N., Valentine, G., Arnold, H.J. (1992). Contribution to the study of the essential oils from three species of *Salvia* growing wild in the eastern Mediterranean region. *Journal of Essential Oil Research*, 4, 607-614. [CrossRef]
 17. Doğan, G., Hayta, Ş., Demirpolat, A., Bağcı, E. (2017). Composition of the essential oil of endemic *Salvia cryptantha* Lamiaceae Montbret & Aucher Ex Benth from Turkey. *Hacettepe Journal of Biology and Chemistry*, 45(3), 315-320. [CrossRef]
 18. Kaya, A., Doğu, S., Demirci, B. (2022). Geographical impact on essential oil composition of endemic *Salvia absconditiflora* collected from different parts of Turkey. *European Journal of Life Sciences*, 1(2), 55-62. [CrossRef]
 19. Demirpolat, A. (2023). Essential oil composition analysis, antimicrobial activities, and biosystematic studies on six species of *Salvia*. *Life*, 13(3), 634. [CrossRef]
 20. Yılar, M., Bayar, Y., Bayar, A.A. (2020). Allelopathic and antifungal potentials of endemic *Salvia absconditiflora* Greuter & Burdet collected from different locations in Turkey. *Allelopathy Journal*, 49(2), 243-256. [CrossRef]
 21. Islam, M.A., Islam, M., Hasan, R., Hossain, M.I., Nabi, A., Rahman, M., Goessens, W.H.F., Endtz, H.P., Boehm, A.B., Faruque, S.M. (2017). Environmental spread of NDM-1-producing multi-drug resistant bacteria in Dhaka, Bangladesh. *Applied and Environmental Microbiology*, 83. [CrossRef]
 22. World Health Organization. (2017). Implementation of the global action plan on antimicrobial resistance. Erişim adresi: <https://www.who.int/>. Erişim tarihi: 17.05.2017.
 23. Uroz, S., Dessaux, Y., Oger, P. (2009). Quorum sensing and quorum quenching: The yin and yang of bacterial communication. *Chembiochem*, 10(2), 205-216. [CrossRef]
 24. Nithya, C., Begum, M.F., Pandian, S.K. (2010). Marine bacterial isolates inhibit biofilm formation and disrupt mature biofilms of *Pseudomonas aeruginosa* PAO1. *Applied Microbiology and Biotechnology*, 88(1), 341-358. [CrossRef]
 25. Lagha, R., Ben Abdallah, F., Al-Sarhan, B.O., Al-Sodany, Y. (2019). Antibacterial and biofilm inhibitory activity of medicinal plant essential oils against *Escherichia coli* isolated from UTI patients. *Molecules*, 24(6), 1161. [CrossRef]
 26. Çiçek Polat, D., Gümüşok, S., Rızvanoğlu, S.S., Eryılmaz, M. (2023). Bioactivities of *Cotinus coggygia* and its HPLC-DAD phenolic profiles, *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, 157(5), 1061-1066. [CrossRef]
 27. Wińska, K., Maćzka, W., Łyczko, J., Grabarczyk, M., Czubaszek, A., Szumny, A. (2019). Essential oils as antimicrobial agents-myth or real alternative? *Molecules*, 24(11), 2130. [CrossRef]
 28. McLafferty, F.W., Stauffer, D.B. (1989). *The Wiley/NBS Registry of Mass Spectral Data*. New York: J Wiley and Sons. p.256.
 29. Hochmuth, D.H. (2008). *MassFinder 4.0*, Hochmuth Scientific Consulting. Hamburg, Germany. p. 1137-1144.
 30. Clinical and Laboratory Standards Institute (CLSI) (2009). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard*. Wayne, PA, USA. p. 15-50.
 31. Bali, E.B., Türkmen, K.E., Erdönmez, D., Sağlam, N. (2019). Comparative study of inhibitory potential of dietary phytochemicals against quorum sensing activity of and biofilm formation by *Chromobacterium violaceum* 12472, and swimming and swarming behaviour of *Pseudomonas aeruginosa* PAO1. *Food Technology and Biotechnology*, 57(2), 212-221. [CrossRef]
 32. Eryılmaz, M., Kart, D., Gürpınar, S.S. (2019). Vajinal floradan izole edilen *Lactobacillus* sp. metabolitlerinin antibiyofilm aktivitelerinin araştırılması. *Türk Mikrobiyoloji Cemiyeti Dergisi*, 49(3), 169-174.
 33. Jardak, M., Mnif, S., Ayed, R.B., Rezgui, F., Aifa, S. (2021). Chemical composition, antibiofilm activities of Tunisian spices essential oils, and combinatorial effect against *Staphylococcus epidermidis* biofilm. *Lebensmittel-Wissenschaft & Technologie*, 140, 110691. [CrossRef]
 34. Gajdács, M., Spengler, G. (2020). Standard operating procedure (SOP) for disk diffusion-based quorum sensing inhibition assays. *Acta Pharmaceutica Hungarica*, 89, 117-125. [CrossRef]

35. Batohi, N., Lone, S.A., Marimani, M., Wani, M.Y., Al-Bogami, A.S., Ahmad, A. (2021). Citral, and its derivatives inhibit quorum sensing and biofilm formation in *Chromobacterium violaceum*. Archives of Microbiology, 203(4), 1451-1459. [\[CrossRef\]](#)
36. Bingöl, Ü., Cosge, B., Ipek, A., Gürbüz, B., Geven, F.G. (2009). Identification of essential oil components of *Salvia cryptantha* Montbret & Aucher ex Benthams, growing wild in Turkey. Asian Journal of Chemistry, 21(5), 3836-3840.
37. İpek, A., Gürbüz, B., Bingöl, M.Ü., Geven, F., Akgül, G., Afshar Pour Rezaeieh, K., Coşge, B. (2012). Comparison of essential oil components of wild and field grown *Salvia cryptantha* Montbert & Aucher ex Benthams, in Turkey. Turkish Journal of Agriculture and Forestry, 36, 668-672. [\[CrossRef\]](#)
38. Bağci, E., Vural, M., Dirmenci, T., Bruehl, L., Aitzetmüller, K. (2004). Fatty acid and tocopherol patterns of some *Salvia* L. species. Zeitschrift für Naturforschung-Section C Journal of Biosciences, 59(5-6), 305-309. [\[CrossRef\]](#)
39. Saadia, Z., Özcan, M.M., Bağci, Y., Ünver, A., Arslan, D., Durak, G., Er, F., Sağlam, C. (2010). Chemical composition of the essential oil of *Salvia cryptantha*. Journal of Essential Oil Bearing Plants, 13(2), 200-204. [\[CrossRef\]](#)
40. Kokoska, L., Kloucek, P., Leuner, O., Novy, P. (2019). Plant-derived products as antibacterial and antifungal agents in human health care. Current Medicinal Chemistry, 26(29), 5501-5541. [\[CrossRef\]](#)
41. Civra, A., Francese, R., Sinato, D., Donalizio, M., Cagno, V., Rubiolo, P., Ceylan, R., Uysal, A., Zengin, G., Lembo, D. (2017). In vitro screening for antiviral activity of Turkish plants revealing methanolic extract of *Rindera lanata* var. *lanata* active against human rotavirus. BMC Complementary Medicine and Therapies, 17(1), 74. [\[CrossRef\]](#)
42. Süntar, I., Akkol, E.K., Senol, F.S., Keles, H., Orhan, I.E. (2011). Investigating wound healing, tyrosinase inhibitory and antioxidant activities of the ethanol extracts of *Salvia cryptantha* and *Salvia cyanescens* using *in vivo* and *in vitro* experimental models. Journal of Ethnopharmacology, 135(1), 71-77. [\[CrossRef\]](#)
43. Tepe, B., Donmez, E., Unlu, M., Candan, F., Daferera, D., Vardar-Unlu, G., Polissiou, M., Sokmen, A. (2004). Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chemistry, 84, 519-525. [\[CrossRef\]](#)
44. Yalcin, A., Yumrutas, O., Kuloglu, T., Elibol, E., Parlar, A., Yilmaz, I., Pehlivan, M., Dogukan, M., Uckardes, F., Aydin, H., Turk, A., Uludag, O., Sahin, İ., Ugur, K., Aydin, S. (2017). Hepatoprotective properties for *Salvia cryptantha* extract on carbon tetrachloride-induced liver injury. Cellular and Molecular Biology (Noisy-le-grand), 63(12), 56-62. [\[CrossRef\]](#)
45. Zhang, H., Li, L., Hao, M., Chen, K., Lu, Y., Qi, J., Chen, W., Ren, L., Cai, X., Chen, C., Liu, Z., Zhao, B., Li, Z., Hou, P. (2021). Yixin-Fumai granules improve sick sinus syndrome in aging mice through Nrf-2/HO-1 pathway: A new target for sick sinus syndrome. Journal of Ethnopharmacology, 277, 114254. [\[CrossRef\]](#)
46. Mączka, W., Duda-Madej, A., Górný, A., Grabarczyk, M., Wińska, K. (2021). Can eucalyptol replace antibiotics? Molecules, 26(16), 4933. [\[CrossRef\]](#)
47. Cai, Z.M., Peng, J.Q., Chen, Y., Tao, L., Zhang, Y.Y., Fu, L.Y., Long, Q.D., Shen, X.C. (2021). 1,8-Cineole: A review of source, biological activities, and application. Journal of Asian Natural Products Research, 23(10), 938-954. [\[CrossRef\]](#)
48. Chen, W., Vermaak, I., Viljoen, A. (2013). Camphor-A fumigant during the Black Death and a coveted fragrant wood in ancient Egypt and Babylon-A Review. Molecules, 18(5), 5434-54. [\[CrossRef\]](#)
49. Alain, K.Y., Tamfu, A.N., Kucukaydin, S., Ceylan, O., Pascal, A.D.C., F'elicien, A., Dominique, S.C.K., Duru, M.E., Mihaela Dinica, R.M. (2022). Phenolic profiles, antioxidant, anti-quorum sensing, antibiofilm and enzyme inhibitory activities of selected *Acacia* species collected from Benin. LWT-Food Science and Technology, 171, 114162. [\[CrossRef\]](#)
50. Tamfu, A.N., Ceylan, O., Cârâc, G., Talla, E., Dinica, R.M. (2022). Antibiofilm and anti-quorum sensing potential of cycloartane-type triterpene acids from cameroonian grassland propolis: Phenolic profile and antioxidant activity of crude extract. Molecules, 27, 4872. [\[CrossRef\]](#)
51. Lahiri, D., Dash, S., Dutta, R., Nag, M. (2019). Elucidating the effect of anti-biofilm activity of bioactive compounds extracted from plants. Journal of Biosciences, 44(2), 52. [\[CrossRef\]](#)
52. Kalia, V.C. (2013). Quorum sensing inhibitors: An overview. Biotechnology Advances, 31, 224-245. [\[CrossRef\]](#)
53. Bhardwaj, A.K., Vinothkumar, K., Rajpara, N. (2013). Bacterial quorum sensing inhibitors: Attractive alternatives for control of infectious pathogens showing multiple drug resistance. Recent Advances in Anti-Infective Drug Discovery, 8, 68-83. [\[CrossRef\]](#)
54. El-Naggar, M.H., Elgaml, A., Abdel Bar, F.M., Badria, F.A. (2019). Antimicrobial and anti-quorum-

- sensing activity of *Ricinus communis* extracts and ricinine derivatives. *Natural Product Research*, 33(11), 1556-1562. [\[CrossRef\]](#)
55. White, C.E., Winans, S.C. (2007). Cell-cell communication in the plant pathogen *Agrobacterium tumefaciens*. *Philosophical Transactions of the Royal Society B*, 362, 1135-1148. [\[CrossRef\]](#)
56. Dickschat, J.S. (2010). Quorum sensing and bacterial biofilms. *Natural Product Reports*, 27, 343-369. [\[CrossRef\]](#)