



## HEALTH SCIENCES

# Composition and bioactivity of essential oil from *Stachys macrostachya* (Wend.) Briq

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**Abstract:** *Stachys* species belonging to Lamiaceae family have been used for medicinal purposes since ancient times. The aim of the present study was to investigate the chemical compositions and antibacterial, anti-tyrosinase activities of the essential oil of *Stachys macrostachya*. The essential oil was prepared by hydrodistillation method using a Clevenger-type apparatus and chemical composition was determined by gas chromatography (GC). The antibacterial activity of essential oil was performed by the disc diffusion and microdilution broth method against five Gram-positive and two Gram-negative bacteria. The tyrosinase inhibitory activity was evaluated by minor modifications of Masuda's method. According to the results of GC analyses, twenty-three compounds were identified representing 91.9% of the total volatile composition. The main compounds were germacrene D (12.2%), globulol (10.9%),  $\alpha$ -pinene (9.7%), and valencene (7.6%). The present study showed that the tested essential oil of *S. macrostachya* exhibited antibacterial activity against *Acinetobacter baumannii* (MIC 62.50  $\mu\text{g/mL}$ ) and tyrosinase inhibition activity ( $\text{IC}_{50}$  22.86  $\pm$  0.82  $\mu\text{g/mL}$ ). These results suggest that the essential oil could be exploited as a potential source of natural antimicrobial agents of this bacterium as well as tyrosinase inhibitors.

**Key words:** *Stachys macrostachya*, *Acinetobacter baumannii*, essential oil, GC/MS, tyrosinase inhibitory activity.

## INTRODUCTION

The genus *Stachys* L. is one of the largest genera of the Lamiaceae (also known as Labiatae) family, and it consists of 370 species (435 taxa) in the world. The genus *Stachys* includes 91 species (118 taxa) under 14 sections in Turkey where is one of the most important gene centers (Akcicek et al. 2012). *Stachys* L. have been used in traditional medicine for the treatment of the common cold (Mukemre et al. 2015), cough (Altundag & Ozturk 2011), diarrhea, urinary system disorders, hypertension, headache, throat pain (Ozdemir & Akpınar 2015), cardiac disorders (Polat et al. 2015). *Stachys* species also have many pharmacological activities including

antipyretic, stomachic (Altundag & Ozturk 2011), anti-inflammatory (Khanavi et al. 2005), anti-anxiety (Rabbani et al. 2003), antibacterial (Grujic-Jovanovic et al. 2004), anti-nephritic (Hayashi et al. 1994), anticancer (Amirghofran et al. 2006), anti-helicobacter (Stamatis et al. 2003), antioxidant (Erdemoglu et al. 2006), and cytotoxic effects (Haznagy-Radnai et al. 2008, Ferhat et al. 2017). Flavonoids, iridoids, fatty acids, and phenolic acids are the main components of this genus (Duru et al. 1999, Ferhat et al. 2017). The essential oil compositions of the *Stachys* genus have been well determined in many studies (Duru et al. 1999, Grujic-Jovanovic et al. 2004, Goren et al. 2011, Goren 2014), but not for all the species have been described in detail. The main

components of the essential oil of this genus were reported to consist of sesquiterpenes and oxygenated sesquiterpenes such as germacrene D, caryophyllenes, cadinene, cubebol, spathuleneol, and caryophyllene (Harmandar et al. 1997, Grujic-Jovanovic et al. 2004, Goren et al. 2011, Goren 2014, Giuliani et al. 2017, El Mokni et al. 2018). The antibacterial effects of these compounds against various bacteria have been studied by many researchers (Goren et al. 2011, Goren 2014, Giuliani et al. 2017).

*Stachys* species are known for its various biological activities. In this context, the presence of tyrosinase inhibitors on various plants is a desirable feature in recent years. As known, melanin is a heterogeneous polyphenol-like biopolymer pigment and is commonly found in bacteria, fungi, plants, and animals (Prota 1988). Tyrosinase is an enzyme that is involved in the synthesis of melanin and is responsible for the coloration of the mammalian skin and hair. The production of high amounts of melanin in the skin causes serious aesthetic problems in humans (Priestley 1993). Excessive melanin accumulation causes a variety of skin disorders, also leads to enzymatic browning of foods, which adversely affects the economy (Kim & Uyama 2005). Therefore, tyrosinase inhibitors have become extremely important in the past few decades (Chang 2009).

In this study, the chemical components of *Stachys macrostachya* (Wend.) Briq essential oil (SMEO) were analyzed by GC systems. In addition, the antibacterial activities against *Acinetobacter baumannii*, *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Streptococcus salivarius* and tyrosinase inhibitory activity of SMEO were tested.

## MATERIALS AND METHODS

### Plant material

The aerial parts of *S. macrostachya* were collected from Konakli Mountain (Erzurum Province, 2403 m, Turkey) in July, 2018. The herbarium sample of the plant was stored at the Herbarium of Ataturk University, Faculty of Pharmacy (AUEF1355).

### Preparation of essential oil

The collected plant was dried in an airy, moisture-free room without direct sunlight. The dried plant was dusted just before starting the extraction to prevent loss of essential oil. The powdered samples (120 g) were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. Once obtained, the essential oil was stored at 4°C for further tests.

### GC/MS and GC/FID conditions.

GC/MS analysis was carried out on an Agilent 7820A gas chromatography system equipped with 7673 series autosampler chemstation and 5977 series mass selective detector. The chromatographic column used for the analysis was an HP-5 MS column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Helium was used as carrier gas at a flow rate of 1.5 mL/ minutes and split ratios 1:40. The injection volume was 1 µL and the injector and detector temperatures were 250 and 300 °C, respectively. Mass spectra were taken at 70 eV with mass range 30 to 450 m/z. The capillary column coated with 5% phenyl and 95% dimethylpolysiloxane for separation of essential oils. The sample (1:100, v/v, in dichloromethane) was injected and analyzed with the column held initially at 60 °C for 10 minutes and then increased to 220 °C with a 4 °C/minutes heating ramp. It was kept in this temperature for 10 minutes. The oven temperature was finally heated to 240 °C at a rate of 1 °C/minutes and kept in this temperature for 80 minutes (Baser et

al. 2000). GC/FID analysis was performed using an Agilent 6890 gas chromatograph combined with a flame ionization detector (FID) and a 7683 autosampler (Agilent Technologies, Palo Alto, CA) system. FID temperature was set to 300 °C and the same conditions were applied to a triplicate of the same column used in GC/MS analyses. Simultaneous auto injection was performed to obtain equivalent retention times. Relative percentages of the separated compounds were calculated from integration of the peak areas in the GC/FID chromatograms.

### Identification of components

The n-alkane series was used as a standard for the determination of essential oil components and the NIST Library, Adams Library (Adams 2007) were used for comparisons of retention indices.

### Bacterial strains

Bacterial strains were five Gram-negative (*A. baumannii*, *E. coli*, *E. faecalis*, *E. faecium*, *P. aeruginosa*) and two Gram-positive bacteria (*S. mutans*, *S. salivarius*). These strains obtained from Molecular Microbiology Laboratory Culture Collection (Erzurum Technical University, Erzurum, Turkey) were suspended in Mueller Hinton Broth (MHB) and incubated for 16-24h at 37 °C.

### Antibacterial activity

#### Disc diffusion method

Bacterial cultures were adjusted to 0.5 McFarland in NaCl 0.9% (or MHB). Agar MH plate surface was inoculated by spreading 100 µL of the bacterial suspension. The sterile paper discs (6 mm in diameter) were impregnated with 10 µL of SMEO (500 µg/mL<sup>-1</sup>) and placed on the inoculated agar. Tetracycline (10 µg/disc) and

streptomycin (25 µg/disc) were used as positive controls. Dimethyl sulfoxide (DMSO) was used as a negative control. Then, these plates were incubated under the suitable conditions which are at 37 °C for 24-72 h. At the end of the incubation, antimicrobial activity was evaluated by measuring the diameter of inhibition zone. The experiment was repeated in triplicates (Gormez et al. 2015).

#### Microdilution method

The antibacterial activity of SMEO was determined by microdilution broth method. Minimum inhibitory concentration (MIC) was expressed as the minimum amount of SMEO that prevented visible bacterial growth after 24 hours of incubation. 0.5 McFarland bacterial suspensions were inoculated in microwell plates to reach a final concentration of  $1.5 \times 10^8$  CFU/mL. Two-fold serial dilutions methodology, of the essential oil provided concentrations ranging from 250 to 3.91 µg/mL. The negative control was prepared as the well containing 195 µL MHB without SMEO and 5 µL of the bacterial inoculate. The positive controls were prepared as the well containing Maxipime (Bristol-Myers Squibb) at the concentration ranging from 250 µg/µL to 3,91 µg/µL in MHB and 5 µL of the bacterial inoculum. The plate was covered with a sterile plate sealer and incubated at 37°C for 24 h. Microbial growth was also monitored at 600 nm after the incubation period (Gormez et al. 2015).

#### Tyrosinase inhibitory activity

Mushroom tyrosinase inhibition activity was determined as described by Masuda's colorimetric method with some modification (Masuda et al. 2005). 3,4-Dihydroxyl-L-phenylalanin (L-DOPA) was used as a substrate while kojic acid (KA) was used as positive

control. 40 µL sample solution was mixed with 40 µL tyrosinase solution (46 U/mL) and 80 µL phosphate buffer (1/15M, pH=6.8) in a 96 well microplate and incubated for 10 minutes at 23°C and 40 µL of L-DOPA (2.5 mM) were put into each well. The absorbance values at 490 nm for each well were measured by a microplate reader (Bio Tek ELx800). All test materials were tested at different concentrations (25, 50, 100, 200 µg/mL). Experiments were performed in 3 replicates. The percent inhibition values were determined, and then  $IC_{50}$  values were calculated by nonlinear regression using GraphPad Prism 8 software. Statistical analyses were also performed using GraphPad Prism 8 software, and p values were calculated by independent samples t test. The percentage of tyrosinase inhibition (I%) was calculated as follows:  $I\% = [(A-B)-(C-D)/A-B] \times 100$ , where A is the absorbance of the control (buffer and tyrosinase), B is the absorbance of the blank (buffer), C is the absorbance of the reaction mixture (buffer, tyrosinase, and sample), and D is the absorbance of the blank of C (buffer and sample).

## RESULTS

### GC/MS analysis

The essential oil was obtained from *S. macrostachya* with a yield of 0.066% (w/w) by hydrodistillation method. The essential oil composition, retention index and retention time of *S. macrostachya* are summarized in Table I. A total of twenty-three components were detected, accounting for 91.9% of the total volatile composition. In this study, the main components were sesquiterpenoids (germacrene D (12.2%), valencene (7.6%), and monoterpenoids (globulol (10.9%), and  $\alpha$ -pinene (9.7%)) of total SMEO.

### Antibacterial activity

In this study, the essential oil at different concentrations was also tested for antibacterial activity against 7 pathogenic bacterial strains. An inhibition zone of above 6 mm in diameter was regarded as a positive result. SMEO exhibited antibacterial activity against only one of the tested bacteria (20 mm inhibition zone) (Fig. 1). 10% DMSO was used as negative control exhibited no inhibition zone. According to microdilution test results, SMEO displayed a MIC value of 62.5 µg/mL against *A. baumannii*. Antimicrobial activity was not observed in tetracycline and streptomycin used as positive controls (Fig. 1).

### Tyrosinase inhibitory activity

In this study, tyrosinase inhibitory activity of SMEO was tested at different concentrations. The percent inhibition effects of all doses of SMEO ( $p < 0.001$ ) were significant when compared with KA (Fig. 2).  $IC_{50}$  value of SMEO ( $IC_{50} 22.86 \pm 0.82$  µg/mL,  $p = 0.021$ ) and positive control KA ( $IC_{50} 3.86 \pm 0.94$  µg/mL) were calculated using percent inhibition values.

## DISCUSSION

The genus *Stachys* having great variety of species is quite rich in essential oils. The sesquiterpenoids in essential oils are known as the major group of substances in all *Stachys* taxa (Skaltsa et al. 2003). Although there are many studies on the chemical contents of *Stachys* species, there is no information on chemical content of the aerial parts of *S. macrostachya* used in this study. The essential oil was obtained from *S. macrostachya* with an efficiency of 0.066% (w/w) by hydrodistillation. Previous studies demonstrated that the essential oil of *Stachys* species contains main components

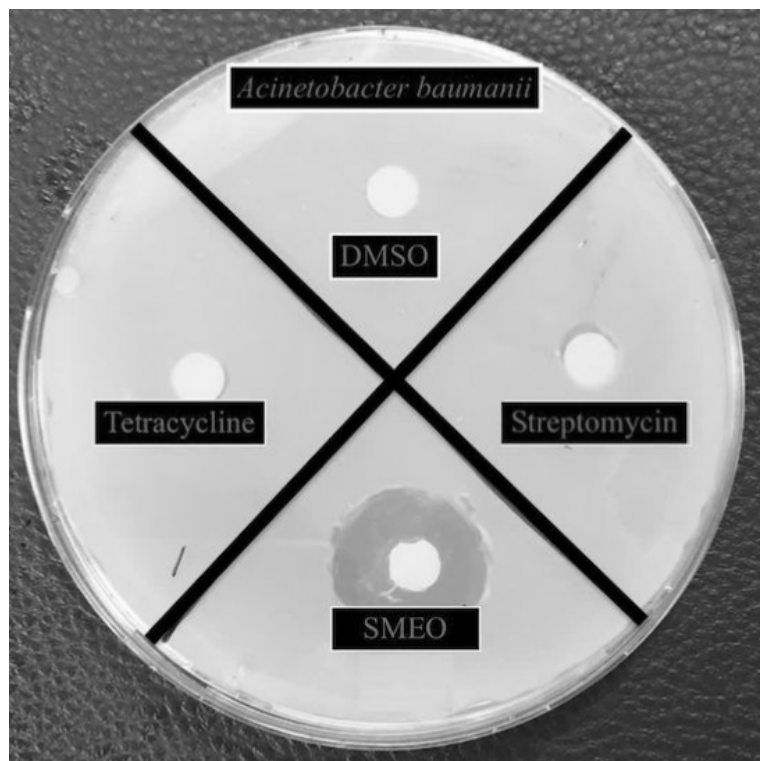
**Table I. Chemical compositions of the essential oil of *S. macrostachya*.**

No	RT	RI	Compounds	Concentration (%)
1	7.29	929	$\alpha$ -pinene	9.72
2	13.00	1031	limonene	1.55
3	28.60	1384	$\beta$ -bourbonene	2.79
4	28.87	1391	$\beta$ -elemene	2.08
5	29.78	1419	(E)-caryophyllene	4.29
6	31.02	1457	$\beta$ -farnesene	2.86
7	31.68	1477	$\gamma$ -muurolene	1.42
8	31.83	1481	germacrene D	12.19
9	31.92	1487	$\alpha$ -amorphene	2.64
10	31.99	1492	$\alpha$ -selinene	3.98
11	32.22	1494	valencene	7.57
12	32.59	1495	levo- $\beta$ -elemene	1.83
13	32.98	1527	$\alpha$ -panasinsen	1.45
14	33.16	1528	$\delta$ -cadinene	1.55
15	34.15	1543	cis-sesquisabinenehydrat	1.78
16	34.92	1576	spathulenol	0.83
17	35.05	1581	caryophyllene oxide	1.24
18	36.45	1586	neryl isovalerate	3.82
19	37.18	1589	isoaromadendrene epoxide	4.17
20	37.63	1591	globulol	10.97
21	37.98	1684	$\alpha$ -bisabolol	5.39
22	42.15	1692	cis-Z- $\alpha$ -bisabolene epoxide	5.47
23	53.17	1792	2,6,10,14-tetramethylhexadecane	2.28
Total				91.9

RT: Retention Time (minute), RI: Retention Index on HP-5 MS column.

structurally related to sesquiterpene such as (E)-caryophyllene, caryophyllene oxide,  $\delta$ -cadinene,  $\alpha$ -curcumene, bicyclogermacrene, germacrene D,  $\alpha$ -humulene, ledol, spathulenol, cis-sesquisabinene hydrate,  $\alpha$ -calacorene, valeranone, as well as substances structurally related to monoterpene such as  $\alpha$ -pinene,  $\beta$ -pinene,  $\beta$ -phellandrene 1,8-cineole, limonene, linalyl acetate, linalool, (-)- $\beta$ -linalool, geranyl acetate, terpinen-4-ol,  $\alpha$ -terpineol, myrtenyl acetate, cis-chrysanthenyl acetate, pinocarvone, and cis-verbenol (Tundis et al. 2014). The major

components of the essential oil of *Stachys* grown in Turkey have been reported as monoterpene hydrocarbons (Renda et al. 2017). At the same time, it was detected that germacrene D was one of the main components in some *Stachys* species such as *S. viticina*, *S. obliqua*, *S. balansae*, *S. sericantha*, *S. pinetorum*, *S. bayburtensis*, *S. huber-morathii*, *S. huetii*, *S. tmolea*, and *S. bithynica* (Goren et al. 2011, Goren 2014). In some previous studies, it was reported that one of the main component of essential oils of *S. balansae* and *S. glutinosa* was  $\alpha$ -pinene, while

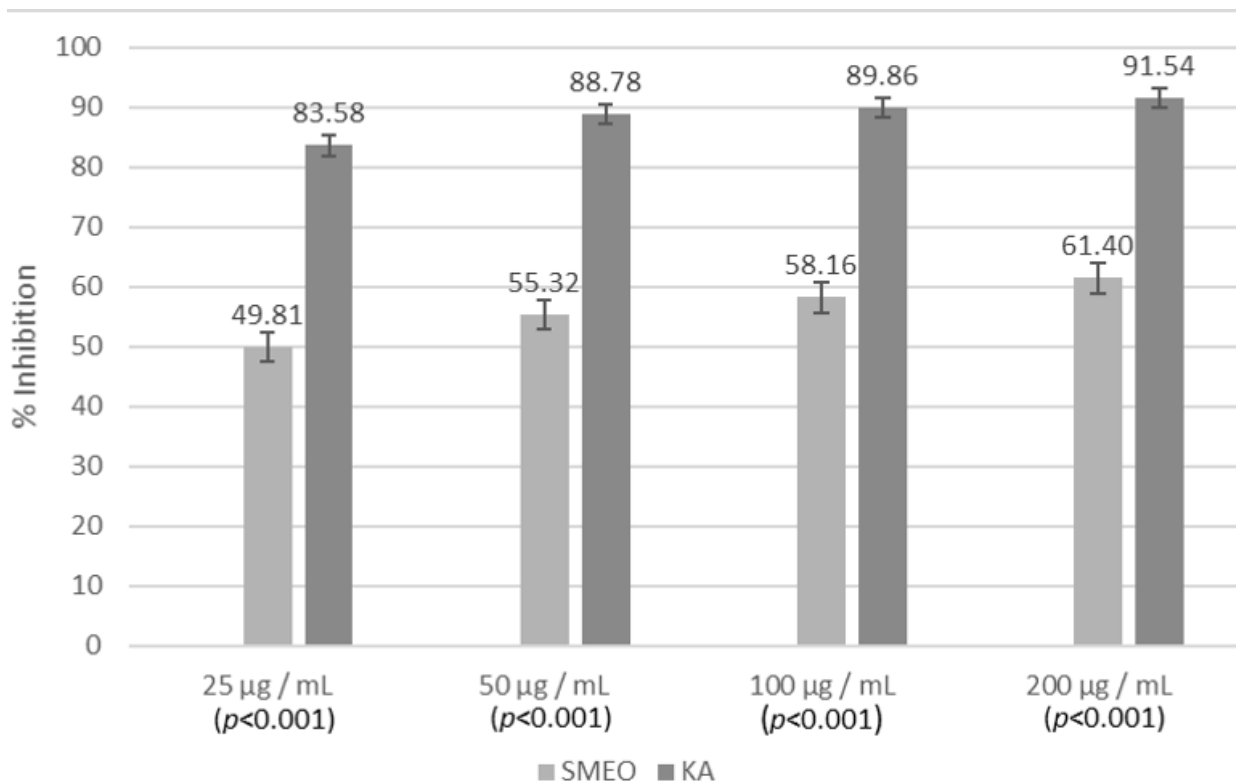


**Figure 1.** The results of antibacterial activity of SMEO against *Acinetobacter baumannii* (Positive controls: Tetracycline and Streptomycin, Negative control: DMSO).

in another study one of the main component of essential oil of *S. circinnata* was globulol (Cakir et al. 1997, Mariotti et al. 1997, Laggoune et al. 2009). Besides, some species such as *S. aleurite*, *S. lavandulifolia*, and *S. schtschegleevii* have been found to contain both  $\alpha$ -pinene and germacrene D as the major components (Javidnia et al. 2004, Flamini et al. 2005, Sonboli et al. 2005). Similarly, the results of our study showed that sesquiterpenoids (germacrene D (12.2%), valencene (7.6%), and monoterpenoids (globulol (10.9%), and  $\alpha$ -pinene (9.7%)) were major components of total SMEO. In this context, our results have been found compatible with all these findings.

Many bacteria causing damage to human health have acquired drug resistance because of excessive and improper use of antibiotics. Thus, there is an urgent need for the discovery of new antimicrobial substance from natural sources including plants. Antimicrobial activities of Lamiaceae plants have been reported in many

studies, but there are a few investigations of antimicrobial activity of *Stachys* species (Grujic-Jovanovic et al. 2004, Saeedi et al. 2008, Dulger & Aki 2009, Goren et al. 2011, Dulger & Dulger 2015). However, to best of our knowledge, there is no report for the antimicrobial activity of *S. macrostachya*. So, this study is the first record to evaluate the antibacterial activity of essential oil of *S. macrostachya*. The efficiencies of the essential oils of some *Stachys* species on various Gram-positive bacteria, Gram-negative bacteria, and fungi have been previously shown (Ozturk et al. 2009, Goren et al. 2011, Lazarević et al. 2013, Yavuz et al. 2017). In this study, the antibacterial effect of SMEO was studied on *A. baumannii*, *E. coli*, *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. mutans*, and *S. salivarius*. The results of this study showed that SMEO possessed antimicrobial activity against only *A. baumannii* from the tested microorganisms. SMEO has shown a significant antimicrobial effect on *A. baumannii*, a multi-drug resistant bacteria



**Figure 2. Tyrosinase inhibition activity of *S. macrostachya*, KA: Kojic acid (Positive control), % Inhibition: The percent inhibition in comparison with a control containing no inhibitor, n= 3, p: test statistics p value of independent samples t test.**

species that endanger the lives of patients hospitalized in intensive care units. *A. baumannii* is an opportunistic and Gram-negative pathogen that leads to serious infections including bacteremia, meningitis, pneumonia, urinary tract infection, wound infection, and even septic shock and death. Therefore, it is very difficult to control and treatment of this bacterium (Winn 2006, Eliopoulos et al. 2008, Dal et al. 2012). It was interesting to find that the SMEO was able to significantly inhibit *A. baumannii*, which is difficult to control and treat. There are a few studies that evaluate the antibacterial activity of different *Stachys* spp. against *A. baumannii* (Mikaili et al. 2011, Alpay et al. 2017), but to the best of our knowledge, this is the first study that evaluates the antibacterial activity of SMEO against this bacterium. The antimicrobial

activities of twenty-two *Stachys* species against *Candida albicans*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *P. aeruginosa*, and *E. coli* have previously been reported by Goren et al. (2011). As a result of the study, most of the essential oils showed moderate activity against the studied microorganisms (Goren et al. 2011). In our study, SMEO was not found effective against other tested microorganisms except *A. baumannii*. The differences in antibacterial activities of plant extracts or essential oils are well known to be due to their components' functional groups, their structural configuration and possible synergistic interactions.

Tyrosinase is a multifunctional, glycosylated, and copper-containing oxidase, which is responsible for production of mammalian melanin pigments and enzymatic browning

reactions of fruits. Hence, natural and non-toxic tyrosinase inhibitors have become increasingly important in medical, food, and cosmetics industry (Chang 2009). In our study, it was investigated to tyrosinase inhibitory effect of essential oil of the aerial parts of *S. macrostachya*. This is the first report demonstrating that the essential oils obtained from *S. macrostachya* was significant tyrosinase inhibitor. It has been previously shown that the tyrosinase inhibitory effects of various extracts of some *Stachys* species. In a previous study, the *n*-hexane, dichloromethane, methanol, ethanol 70%, and methanol extracts of *S. lavandulifolia* aerial parts were tested for their potential tyrosinase inhibitory activity and it was reported that 70% ethanol and methanol extracts inhibited tyrosinase with IC<sub>50</sub> values of 33.4 and 42.8 µg/mL, respectively (Tundis et al. 2015). In other studies, the tyrosinase inhibitory effects of water, methanol, and ethyl acetate extracts of the aerial parts of *Stachys cretica* subsp. *vacillans*, *S. annua* (L.) subsp. *annua*, *S. byzanthina*, and *S. cretica* subsp. *mersinaea* were analyzed. Interestingly, they have reported the strongest anti-tyrosinase activities for ethyl acetate extracts of different *Stachys* species (Sarikurkcu et al. 2016, Kocak et al. 2017, Bahadori et al. 2019, Kirkan 2019). Ethyl acetate extract is generally rich in phenolic compounds and flavonoids and these compounds are known to be good tyrosinase inhibitors (Chang 2009). In our study, the tyrosinase inhibitory effect of SMEO may be due to any of the compounds in the essential oil or synergistic effects of these compounds.

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#### Author contributions

ESK and AG designed the study. ESK obtained essential oil from the plant. Enzyme study was carried out by ESK and UO, antimicrobial activity studies by AG and FNK, GC analysis by BY. All authors contributed to the paper writing.

