# Chemical and biological profiles of essential oil from different parts of *Myrtus communis* L. subsp. *communis* from Turkey

Ali SEN[1] (✉)
Mine KURKCUOGLU[2]
Aybeniz YILDIRIM[1,3]
Ahmet DOGAN[4]
Leyla BITIS[1]
Kemal HUSNU CAN BASER[2,5]

## Summary

The present study reports chemical composition, antidiabetic, anti-inflammatory and antioxidant potential of essential oils from leaves and twigs of *Myrtus communis* L. subsp. *communis* from Turkey. Essential oils were obtained separately from leaves and twigs of *Myrtus communis* subsp. *communis* (MC) by hydrodistillation using a Clevenger-type apparatus. Chemical compositions were determined using GC/MS. Antidiabetic, anti-inflammatory and antioxidant activities of essential oils were tested by α-amylase inhibitory, 5-lipoxygenase inhibitory and DPPH/ABTS radical scavenging methods, respectively. The major compounds of essential oil of *Myrtus communis* subsp. *communis* leaves (MCLEO) were α-pinene (35.6%), 1,8-cineole (28.3%), linalool (10.5%), and limonene (8.2%), while the major constituents of essential oil of *Myrtus communis* subsp. *communis* twigs (MCTEO) were α-pinene (30.7%), 1,8-cineole (23.5%), *p*-cymene (13.3%) and limonene (11.9%). MCLEO and MCTEO showed good and moderate radical scavenging activity with $IC_{50}$ values of 124.40 µg/mL and 390.10 µg/mL for ABTS radical, respectively. MCLEO and MCTEO exhibited significant radical scavenging activity with $IC_{50}$ values of 34.13 µg/mL and 28.15 µg/mL for DPPH radical, respectively. Also, MCLEO and MCTEO displayed strong and good antidiabetic activity with $IC_{50}$ values of 29.94 µg/mL and 159.80 µg/mL against α-amylase enzyme, respectively. Finally, MCLEO and MCTEO showed good anti-inflammatory activity with $IC_{50}$ values 86.10 µg/mL and 96.55 µg/mL against 5-lipoxygenase enzyme, respectively. From the present study it can be concluded that essential oils, especially MCLEO, possess good antidiabetic, anti-inflammatory and antioxidant activities. Also, this is the first study on chemical composition of MCTEO from Turkey, as well as on α-amylase inhibitory and 5-lipoxygenase inhibitory activities of MCLEO and MCTEO.

## Key words

antidiabetic activity, anti-inflammatory activity, antioxidant activity, essential oil, *Myrtus communis* subsp. *communis*

[1] Department of Pharmacognosy, Faculty of Pharmacy, Marmara University, Istanbul, Turkey
[2] Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey
[3] Adıyaman University, Faculty of Pharmacy, Department of Pharmacognosy, Adiyaman, Turkey
[4] Department of Pharmacetical Botany, Faculty of Pharmacy, Marmara University, Istanbul, Turkey
[5] Department of Pharmacognosy, Faculty of Pharmacy, Near East University, Nicosia, N. Cyprus

✉ Corresponding author: ali.sen@marmara.edu.tr

Received: November 27, 2019 | Accepted: December 19, 2019

## Introduction

The genus *Myrtus* is an aromatic evergreen shrub in the family Myrtaceae and it has solid, leather-likely, small, straight edged, dark green lanceolate leaves (Davis, 1982). *Myrtus* is a medium size genus which includes three accepted taxa in *ca*. 100 taxa, found mostly in the tropics and subtropics, though none is indigenous to tropical Africa (Powo, 2019). *Myrtus communis* L. grows mainly in the Mediterranean phytogeographic region including Turkey. *M. communis* is known by vernacular names "yaban mersini" and "murt" in Turkey. Leaves and fruits of *M. communis* have been used in the treatment of diabetes, hypertension, peptic ulcers, urethritis, conjunctivitis, hemorrhage, wound, skin diseases and respiratory diseases in Turkey (Tuzlacı, 2016). It has been reported by scientific researches that essential oils, extracts or isolated compounds of MC have anti-oxidant (Hayder et al., 2004; Maggio et al., 2019; Mimica-Dukic et al., 2010; Sen et al., 2016; Sen et al., 2017; Wannes et al., 2010a), anti-microbial (Aboutabl et al., 2011), anti-genotoxic (Hayder et al., 2004), anti-mutagenic (Mimica-Dukic et al., 2010), anti-hyperglycaemic (Nassar et al., 2010), anti-inflammatory (Nassar et al., 2010; Sen et al., 2017), anti-cancer (Aboutabl et al., 2011), anti-nociceptive (Nassar et al., 2010), anti-wormal (Aboutabl et al., 2011), neuroprotective (Aykac et al., 2019; Maggio et al., 2019), anti-fibrotic (Sen et al., 2016), and burn wound healing properties (Ozcan et al., 2019).

Previous studies on phytochemical analysis of MC indicated the presence of acylphloroglucinols (Feisst et al., 2005), essential oil (Table 1), catechin (Wannes et al., 2010a), galloyl derivatives (Wannes et al., 2010a), flavonoids (Nassar et al., 2010; Wannes et al., 2010a), and phenolic acids (Nassar et al., 2010; Wannes et al., 2010a). Several studies reported that essential oil of leaves and twigs of MC generally contained $\alpha$-pinene, limonene, 1,8-cineole, linalool, $\alpha$-terpineol, myrtenyl acetate as major components (Table 1). To the best of our knowledge, there is no study on chemical composition of the essential oil from twigs and on the $\alpha$-amylase and 5-lipoxygenase inhibitory activities of essential oils from leaves and twigs of MC. The main goal of the present study was to investigate chemical composition, antidiabetic, anti-inflammatory and antioxidant potential of essential oil from leaves and twigs of *Myrtus communis* L. subsp. *communis* from Turkey.

## Material and methods

### Plant material

Leaves and twigs of *Myrtus communis* subsp. *communis* were collected in the flowering periods from the Antalya province of Turkey in 2018 and identified by Dr. Ahmet Dogan, a botanist of the Faculty of Pharmacy, University of Marmara. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Marmara University (MARE No: 22260).

### Isolation of essential oil

Leaves (60.9 g) and twigs (121.34 g) of *Myrtus communis* subsp. *communis* were separately hydrodistilled for four hours using Clevenger apparatus. The essential oil obtained was stored at 4°C in the dark until analyzed.

### Analysis of the essential oil

The oil was analyzed by capillary GC and GC/MS using a Agilent GC-MSD system.

### Gas Chromatography (GC) and Gas Chromatography - Mass Spectrometry (GC/MS) analysis

*GC-MS conditions:* The oil was analyzed by capillary GC/MS using an Agilent GC-MSD system (Agilent Technologies Inc., Santa Clara, CA). HP-Innowax FSC column (Hewlett-Packard-HP, U.S.A.) (60 m × 0.25 mm i.d., with 0.25 µm film thickness) was used for separation of components in the oil and helium as a carrier gas (0.8 mL/min). The GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°Cmin, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. The split flow was adjusted at 40 mL $min^{-1}$ with 40:1 split ratio. The injector temperature was set at 250°C. Mass spectra were taken at 70 eV with the mass range *m/z* 35-450.

*GC conditions:* The GC analysis was done with Agilent 6890N GC system fitted with a FID detector set at a temperature of 300°C. To obtain the same elution order with GC–MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms by using Agilent ChemStation Plus® software with peak integration process (Kunduhoglu et al., 2011).

### Identification of compounds

Identification of essential oil components were performed by comparison of their mass spectra with those in the *in-house* Baser Library of Essential Oil Constituents, Wiley GC/MS Library, Adams Library, MassFinder Library and confirmed by comparison of their retention indices. A homologous series of *n*-alkanes were used as the reference points in calculation of relative retention indices (RRI). The relative percentages of the separated compounds were calculated from FID chromatograms. Sample of the oil was tested three times. The analysis results are expressed as mean percentage as listed in Table 2.

### Antioxidant activity

#### *2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity*

Free radical scavenging capacity of essential oil were evaluated according to the previously reported procedure using the stable DPPH (Zou et al., 2011). Briefly, 10 µL of essential oil in DMSO at different concentrations (125-0.24 µg/mL) were added to 190 µL methanol solution of DPPH (0.1 mM) in a well of 96-well plate. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. Absorbance readings were taken at 517 nm. The percentage of radical scavenging activity of extracts and standard against DPPH were calculated according to the following:

$$\text{DPPH radical-scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

**Table 1.** Previous studies on chemical composition of *Myrtus communis* leaves and stem essential oil

| Plant part | % Yield | Main compounds (≥5%) | References |
|---|---|---|---|
| Leaves | 3.5 | *α*-pinene (25.5%), 1,8-cineole (27.2%), linalool (11.8%), *p*-menth-1-enol (7.0%) | (Aboutabl et al., 2011) |
| Leaves | 0.10-0.59 | *α*-pinene (45.8%), limonene (5.0%), 1,8-cineole (30.7%) | (Bazzali et al., 2012) |
| Leaves | 0.3 | *α*-pinene (46.9%), 1,8-cineole (25.2%), linalool (5.6%) | (Brada et al., 2012) |
| Leaves | 0.35 | 1,8-cineole (26.91%), *α*-pinene (22.02%), linalool (12.74%), linalyl acetate (8.64%), *α*-terpineol (8.29%) | (Dejam et al., 2017) |
| Leaves | 0.77-0.92 | **α**-pinene (33.4-39.3%), 1,8-cineol (42.4-33.3%), linalool (1.0-6.5%) | (Foudil-Cherif et al., 2013) |
| Twigs | 0.05 | *α*-pinene (10.8%), *p*-cymene (17.7%), limonene (10.7%), 1,8-cineol (13.5%) | |
| Leaves | 0.66-0.69 | *α*-pinene (10.8-21.1%), 1,8-cineol (41.8-49.8%), linalool (20.3-25.2%), linalool acetate (5.0-5.9%) | (Gavahian et al., 2013) |
| Leaves | 0.28 | *α*-pinene (23-49%), 1,8-cineole (10-24%), limonene (11-30%), linalool (2-32%) | (Hennia et al., 2016) |
| Leaves | 0.68 | 1,8-cineole (26.5%), linalool (18.0%), *α*-pinene (11.6%), *α*-terpineol (8.9%) | (Khan et al., 2014) |
| Leaves | 0.08 | *α*-pinene (10.5-57.9%), limonene (3.7-15.1%), 1,8-cineole (7.7-33.3%), linalool (3.7-38.4%), myrtenyl acetate (0.6-28.9%) | (Maggio et al., 2019) |
| Leaves | 0.72-0.81 | *α*-pinene (14.7-35.9%), 1,8-cineole (25.7-23.9%), linalool (10.1-10.9%), myrtenyl acetate (21.6-5.4%) | (Mimica-Dukic et al., 2010) |
| Leaves | - | *α*-pinene (25.53%), 1,8-cineol (27.19%), linalool (11.75%), *p*-menth-1-enol (6.95%) | (Nassar et al., 2010) |
| Leaves | 1.3-2.61 | *α*-pinene (23.0-22.1%), limonene (17.8-17.6%), 1,8-cineole (20.3-24.0%), linalool (12.3-11.4%) | (Pezhmanmehr et al., 2010) |
| Leaves | 0.47-1.87 | *α*-pinene (7.04-31.29%), *β*-myrcene (1.35-6.52%), -limonene (14.02-22.52%), *α*-terpinolene (0.64-8.51%), *α*-terpineol (0.11-5.28%), linalool (1.72-15.47%), 3-cyclohexene-1-methanol (17.31%) | (Rahimi et al., 2015) |
| Leaves | 0.53-1.75 | *α*-pinene (12.2-60.4%), 1,8-cineole (6.2-27.3%), limonene (1.4-26.7%), linalool (0.7-21.0%), myrtenyl acetate (0-29.1%), geraniol (0.3-8.3%) | (Shahbazian et al., 2018) |
| Leaves | 1.2-3.2 | *α*-pinene (1.30-26.76%), limonene (1.94-18.13%), 1,8-cineole (4.49-29.60%), linalool (7.76-29.08%), *α*-terpineol (3.84-24.14%), bornyl-acetate (2.17-8.26%), geranyl-acetate (2.14-15.31%), methyl-eugenol (0.78-5.11%), dihydroeugenyl-butanoate (2.63-11.47%), dihydroeugenyl-pentanoate (0.22-6.82%) | (Usai et al., 2015) |
| Leaves | 0.61 | *α*-pinene (58.05%), *β*-pinene (6.45%), 1,8-Cineole (21.67%) | (Wannes et al., 2010a) |
| Stem | 0.08 | *α*-pinene (10.53%), 1,8-cineole (32.84%), *E*-*β*-ocimene (9.48%), linalool (6.88%) | |
| Leaves | 0.14-0.61 | *α*-pinene (28.3-58.0%), 1,8-cineole (12.7-30.7%), linalool (2.4-21.5%), limonene (0.1-13.3%) | (Wannes et al., 2010b) |
| Stem | 0.001-0.06 | 1,8-cineole (21.0-52.4%), linalool (3.1-18.4%), *α*-pinene (1.5-16.1%) | |

where $A_0$ is the absorbance of the control (containing all reagents except the test compounds), and $A_1$ is the absorbance of the extracts/standard. Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph plotting inhibition percentage against extracts concentration. Tests were carried out in triplicate. Butylated hydroxyanisole, ascorbic acid and trolox were used as positive control.

***2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical-scavenging activity***

Free radical scavenging capacity of essential oil was evaluated according to the previously reported procedure (Zou et al., 2011). ABTS radical cations were prepared by mixing equal volume of ABTS (7 mM in $H_2O$) and potassium persulfate (4.9 mM in $H_2O$), allowing them to react for 12-16 h at room temperature in the dark. Then, ABTS radical solution was diluted with 96% ethanol to an absorbance of about 0.7 at 734 nm. The mixture of 10 µL of essential oil in DMSO at different concentrations (125-0.24 µg/mL) was added to 190 µL of ABTS radical solution in a well of 96-well plate. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. Absorbance readings were taken at 734 nm. The percentage of radical scavenging activity of extracts and standard against ABTS were calculated according to the following:

$$\text{ABTS radical-scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where $A_0$ is the absorbance of the control (containing all reagents except the test compounds), and $A_1$ is the absorbance of the extracts/standard. Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph plotting inhibition percentage against extracts concentration. Tests were carried out in triplicate. Butylated hydroxyanisole, ascorbic acid and trolox were used as positive control.

**Table 2.** Essential oil composition of leaves (MCLEO) and twigs (MCTEO) of *Myrtus communis* L. subsp. *communis*

| RRI | Compounds | MCLEO % | MCTEO % | IM |
|---|---|---|---|---|
| 1032 | *α*-Pinene | **35.5** | **30.7** | $t_R$, MS |
| 1100 | Isobutyl isobutyrate | 0.4 | - | MS |
| 1118 | *β*-Pinene | 0.2 | - | $t_R$, MS |
| 1159 | *δ*-3-Carene | 0.1 | - | $t_R$, MS |
| 1174 | Myrcene | 0.1 | - | $t_R$, MS |
| 1203 | Limonene | **8.2** | **11.9** | $t_R$, MS |
| 1213 | 1,8-Cineole | **28.3** | **23.5** | $t_R$, MS |
| 1246 | (*Z*)-*β*-Ocimene | 0.1 | - | $t_R$, MS |
| 1255 | *γ*-Terpinene | 0.3 | - | $t_R$, MS |
| 1266 | (*E*)-*β*-Ocimene | 0.1 | - | $t_R$, MS |
| 1280 | *p*-Cymene | 0.5 | **13.3** | $t_R$, MS |
| 1290 | Terpinolene | 0.3 | - | $t_R$, MS |
| 1450 | *trans*-Linalool oxide (Fur.) | 0.1 | - | MS |
| 1553 | Linalool | **10.5** | 4.0 | $t_R$, MS |
| 1565 | Linalyl acetate | 1.1 | 0.8 | $t_R$, MS |
| 1571 | Methyl citronellate | 0.1 | - | MS |
| 1611 | Terpinen-4-ol | 0.3 | - | $t_R$, MS |
| 1612 | *β*-Caryophyllene | 0.1 | - | $t_R$, MS |
| 1617 | Hotrienol | 0.1 | - | MS |
| 1670 | *trans*-Pinocarveol | 0.3 | - | $t_R$, MS |
| 1687 | *α*-Humulene | 0.2 | - | $t_R$, MS |
| 1688 | Methyl chavicol | 0.3 | - | MS |
| 1700 | *p*-Mentha-1,8-dien-4-ol | tr | 0.5 | MS |
| 1704 | *α*-Terpinyl acetate | 2.4 | 2.5 | $t_R$, MS |
| 1706 | *α*-Terpineol | 4.4 | 3.1 | $t_R$, MS |
| 1726 | Germacrene D | tr | - | MS |
| 1733 | Neryl acetate | 0.2 | - | $t_R$, MS |
| 1765 | Geranyl acetate | 1.4 | 3.3 | $t_R$, MS |
| 1772 | Citronellol | 0.1 | - | $t_R$, MS |
| 1797 | Myrtenol | tr | - | MS |
| 1808 | Nerol | 0.2 | - | $t_R$, MS |
| 1845 | *trans*-Carveol | 0.2 | - | $t_R$, MS |

| RRI | Compounds | MCLEO % | MCTEO % | IM |
|---|---|---|---|---|
| 1857 | Geraniol | 1.8 | 0.9 | $t_R$, MS |
| 2008 | Caryophyllene oxide | 0.1 | 2.6 | $t_R$, MS |
| 2029 | Methyl eugenol | 0.5 | - | MS |
| 2069 | 3,3,5,5,8,8- Hexamethyl-7-oxabicyclo (4.3.0) non-1 (6)-ene-2,4-dione | 1.3 | - | MS |
| 2071 | Humulene epoxide-II | 0.2 | 2.7 | MS |
| | **Total identified compounds (%)** | **100** | **99.8** | |
| | Monoterpene hydrocarbons | 45.4 | 55.9 | |
| | Oxygenated monoterpenes | 46.5 | 32 | |
| | Sesquiterpenes hydrocarbons | 0.3 | - | |
| | Oxygenated sesquiterpenes | 0.3 | 5.3 | |
| | Others | 7.5 | 6.6 | |

RRI: Relative retention indices experimentally calculated against n-alkanes; % calculated from FID data; tr: Trace (<0.1 %); IM: Identification Method: $t_R$: Identification based on comparison with co-injected with standards on a HP Innowax column; MS: identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries

## Anti-inflammatory activity

### *Antilipoxygenase activity*

The anti-inflammatory activity of oil was evaluated according to the method described by Phosrithong et al. (2016). An aliquot of 500 µL (at different concentrations) of essential oil was added to 250 µL of 0.1 M borate buffer pH 9.0 containing 0.005% Tween 20, followed by addition of 250 µL of type V soybean lipoxygenase solution in buffer (20.000 U/mL). After the mixture was incubated at 25°C for 5 min, 1000 µL of 0.6 mM linoleic acid solution was added, mixed well and the change in absorbance at 234 nm was recorded for 6 min. Indomethacin was used as a reference standard. The percentage of inhibition was calculated from the following equation:

$$\% \text{ inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

A dose-response curve was plotted to determine the $IC_{50}$ values. $IC_{50}$ is defined as the concentration sufficient to obtain 50% of a maximum anti-inflammatory activity. All tests and analyses were performed in triplicates.

## Antidiabetic activity

### *α-amylase inhibitory activity*

The α-amylase inhibitor activity was evaluated with slightly modified method of Ramakrishna et al. (2017). The method was adapted to a 96-well microplate format. A mixture of 10 µL of essential oil, 15 µL of 0.02 M sodium phosphate buffer (pH 6.9, 0.006 M NaCl) and 25 µL of porcine-amylase (0.5 mg/mL-15 units) was prepared in buffer. The mixture was incubated at 25°C for 10 minutes. Then, 25 µL of a 1% starch solution prepared in buffer was added to each well. The mixtures were again incubated at 25°C for 10 minutes. The reaction was stopped with 50 µL of dinitrosalicylic acid (DNSA) and incubated in boiling water bath for 10 minutes. The solutions were cooled to room temperature, diluted with 225 µL of ultra pure water and the absorbance was read at 540 nm. Acarbose was used as standard. The percentage of inhibitory activity of the oil and standard against α-amylase enzyme were calculated according to the following:

$$\alpha\text{-amylase inhibitory activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where $A_0$ is the absorbance of the control (containing all reagents except the test compounds), and $A_1$ is the absorbance of the oil/standard. Essential oil or standard concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph plotting inhibition percentage against oil or standard concentration. Tests were carried out in triplicate.

## Statistical analysis

The data were given as means ± standard deviations and analysed by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison tests using GraphPad Prism 5. Differences between means at $p<0.05$ levels were considered significant.

## Results and discussion

### Essential oil composition

In the current study, yields of light yellow-colored essential oils obtained from essential oil from leaves and twigs of MC were found to be 1.31% and 0.15%, respectively. In previous reports, it was stated that the yield of essential oil obtained from the leaves of MC essential oil ranged from 0.08-3.2% (Table 1). The yield of MC leaf essential oil fell within the yield limits of previous studies. The yield of MC twig essential oil was 0.15%, what was higher than those obtained by Wannes et al.(2010a, 2010b), and Foudil-Cherif et al. (2013) with 0.08, 0.001 - 0.06 and 0.05%, respectively (Table 1).

The results obtained by GC and GC/MS analysis of the MCLEO and MCTEO are presented in Table 2. Thirty seven and thirteen constituents, which represent 100% (MCLEO) and 99.8% (MCTEO) of the total essential oils, respectively, were identified. The MCLEO was characterized by a high content of α-pinene (35.6%), 1,8-cineole (28.3%), linalool (10.5%), and limonene (8.2%), while the predominant compounds in MCTEO were α-pinene (30.7%), 1,8-cineole (23.5%), *p*-cymene (13.3%) and limonene (11.9%) (Table 2). The same major components of the MCLEO were also found in previous studies (Table 1). There is no study on the chemical composition of essential oil of twig of MC collected from Turkey, but there are reports on the chemical composition of essential oil of twig of MC collected from different countries in the literature (Table 1). Particularly, in a study by Foudil-Cherif et al. (2013) on the chemical composition of twig essential oil of MC, the major components of the oil [α-pinene (10.8%), *p*-cymene (17.7%), limonene (10.7%), 1,8-cineole (13.5%)] were found to be compatible with our study. However, other major constituents, except *p*-cymene, were found in higher yields in our study.

The terpenoids were the main portion of MCLEO and MCTEO. In MCLEO, oxygenated monoterpenes formed 46.5%, followed by monoterpene hydrocarbons (45.4%), other compounds (7.5%), sesquiterpenes hydrocarbons and oxygenated sesquiterpenes (both 0.3%). In MCTEO, monoterpene hydrocarbons represented 55.9%, followed by oxygenated monoterpenes (32%), other compounds (6.6%) and oxygenated sesquiterpenes (5.3%) (Table 2). Previous studies on the chemical composition of essential oils obtained from leaves and twigs of MC by researchers from different countries revealed that oils were rich in monoterpenes (Table 1). These results are similar to our study.

### Antioxidant activity of MCLEO and MCTEO

The antioxidant evaluation showed that MCLEO and MCTEO exhibited significant radical scavenging activity with $IC_{50}$ values of 34.13 µg/mL and 28.15 µg/mL, respectively, against DPPH radical, while MCLEO and MCTEO exhibited good and moderate radical scavenging activity with $IC_{50}$ values of 124.40 µg/mL and 390.10 µg/mL, respectively, against ABTS radical (Table 3). Wannes et al. (2010a) reported that essential oils of *Myrtus communis* var. *italica* leaf and stem exhibited low activity against DPPH radical, with $IC_{50}$ values of 600 µg/mL and 2000 µg/mL, respectively. Similarly, Mimica-Dukic et al. (2010) found that the leaf essential oils of MC collected from two different regions in Montenegro had a weak DPPH radical scavenging activity with $IC_{50}$ values of 6240 µg/mL and 5990 µg/mL. In another study, Maggio et al. (2019) reported that the essential oils of *M. communis* leaves collected from nine different regions in Sicily (Italy) showed antioxidant activity against DPPH radical with $IC_{50}$ values in the range 71.4 - 270.8 µg/mL. When we compared these results, MCLEO and MCTEO in our study showed a better antioxidant activity. Previous studies also revealed that α-pinene (Dai et al., 2013), limonene (Dai et al. 2013), 1,8-cineole (Moghadam et al., 2015), linalool (Duarte et al., 2016), *p*-cymene (de Oliveira et al., 2015) possess antioxidant activities. These compounds were found to be major compounds of MCLEO and MCTEO in the present study. Therefore, these compounds together with other compounds may be responsible for the antioxidant activity of the essential oils.

### Antidiabetic activity of MCLEO and MCTEO

The α-amylase inhibitor assay was used to evaluate the the antidiabetic activity at various concentrations of the oil. Results showed that MCLEO and MCTEO have significant and good antidiabetic effect with $IC_{50}$ values of 29.94 µg/mL and 159.80 µg/mL, respectively, against α-amylase enzyme (Table 3). To the best of our knowledge, there is no work on anti-α-amylase activity of MCLEO and MCTEO. However, in studies performed on alloxan-diabetic rabbits and streptozotocin-induced diabetic rats MC leaf essential oil showed a significant antidiabetic effect (Karimlar et al., 2019; Sepici et al., 2004). Karimlar et al. (2019) reported that the essential oil obtained from the leaves of MC showed antidiabetic activity against α-glucosidase enzymes with $IC_{50}$ value of less than 100 µg/mL. These results confirm that the essential oils of MC have antidiabetic activity. There are reports that α-pinene (Özbek et al., 2017), limonene (Murali et al., 2012; More et al., 2014; Habtemariam et al., 2017), linalool (More et al., 2014), *p*-cymene (Habtemariam et al., 2017) that are major compounds of MCLEO and MCTEO in our study, have antidiabetic activity according to the literature. Thus, the present results show that the antidiabetic activity of MCLEO and MCTEO can be attributed to the synergistic activities of monoterpene compounds such as α-pinene, limonene, linalool and *p*-cymene.

### Anti-inflammatory activity of MCLEO and MCTEO

MCLEO and MCTEO were found to have good anti-inflammatory activity with $IC_{50}$ values of 86.10 µg/mL and 96.55 µg/mL, respectively, against 5-lipoxygenase enzyme (Table 3). To our knowledge, this is the first study that has examined the anti-inflammatory effect of MCLEO and MCTEO on lipoxygenase activity. However, there are studies in the literature evaluating the antiinflammatory activity of MC essential oil on different models of inflammation. In one of these studies, Bouzabata et al. (2015) investigated anti-inflammatory potential of MC aerial parts essential oil using an *in vitro* model of lipopolysaccharide stimulated macrophages and they found that oil was able to significantly inhibit nitric oxide production, without affecting cell viability, in concentrations up to 0.64 mg/mL. In another study, Maxia et al. (2011) evaluated the topical anti-inflammatory activity of the essential oil of MC leaves in rats using croton oil induced ear edema and they reported that the essential oil of MC leaves reduced leukocyte migration to the damaged tissue and exhibited antiinflammatory activity. In addition, α-pinene (Özbek

**Table 3.** Antidiabetic, anti-inflammatory and antioxidant activity of essential oil of leaves (MCLEO) and twigs (MCTEO) of *Myrtus communis* L. subsp. *communis*

| Essential oils / Standards | Anti-inflammatory activity | Antidiabetic activity | Antioxidant activity | |
|---|---|---|---|---|
| | Anti-lipoxgenase activity | $\alpha$-amylase inhibitory activity | ABTS radical scavenging activity | DPPH radical scavenging activity |
| | $IC_{50}$ (µg/mL) | | | |
| MCLEO | 86.10 ± 2.80[b] | 29.94 ± 2.77[b] | 124.40 ± 2.69[b] | 34.13 ± 0.83[c] |
| MCTEO | 96.55 ± 2.07[c] | 159.80 ± 2.48[c] | 390.10 ± 0.00[c] | 28.15 ± 1.13[b] |
| Indomethacin | 22.39 ± 0.26[a] | | | |
| Acarbose | | 11.6 ± 0.18[a] | | |
| Trolox | | | 21.44 ± 1.61[a] | |
| Ascorbic acid | | | | 18.0 ± 0.40[a] |

* Each value in the table is represented as mean ± SD (n = 3). Different letter superscripts in the same column indicate significant differences ($P < 0.05$)

et al., 2017), limonene (Yu et al., 2017), 1,8-cineole (Juergens et al., 2014), linalool (Peana et al., 2002) and *p*-cymene (Lotfi et al., 2015) have been reported to possess anti-inflammatory properties. Therefore, these monoterpene compounds, found as major compounds in the essential oil of MCLEO and MCTEO, may be responsible for the anti-inflammatory activity of the oils.

## Conclusion

The present study indicated that MCLEO and MCTEO were rich in monoterpene compounds. It was also found that essential oils, particularly MCLEO, have good antidiabetic, anti-inflammatory and antioxidant activities. These essential oils can be used as a natural source in food, pharmaceutical and cosmetic industries. However, further studies, such as *in vivo* tests, are needed to clarify the antioxidant, antidiabetic and anti-inflammatory effects of these essential oils.

## Conflict of interest

No potential conflict of interest was reported by the authors.

## References


Aboutabl E.A., Meselhy K.M., Elkhreisy E.M., Nassar M.I., Fawzi R. (2011). Composition and bioactivity of essential oils from leaves and fruits of *Myrtus communis* and *Eugenia supraxillaris* (Myrtaceae) grown in Egypt. J Essent Oil Bear Pl 14(2): 192-200.

Aykac A., Ozbeyli D., Uncu M., Ertaş B., Kılınc O., Şen A., Orun O., Sener G. (2019). Evaluation of the protective effect of *Myrtus communis* in scopolamine-induced Alzheimer model through cholinergic receptors. Gene 689: 194-201.

Bazzali O., Tomi F., Casanova J., Bighelli A. (2012). Occurrence of C8–C10 esters in Mediterranean *Myrtus communis* L. leaf essential oil. Flavour Fragr J 27(5): 335-340.

Bouzabata A., Cabral C., Gonçalves M.J., Cruz M.T., Bighelli A., Cavaleiro C., Casanova J., Tomi F., Salgueiro L. (2015). *Myrtus communis* L. as source of a bioactive and safe essential oil. Food Chem Toxicol 75: 166-172.

Brada M., Tabti N., Boutoumi H., Wathelet J.P., Lognay G. (2012). Composition of the essential oil of leaves and berries of Algerian myrtle (*Myrtus communis* L.). J Essent Oil Res 24(1): 1-3.

Dai J., Zhu L., Yang L., Qiu J. (2013). Chemical composition, antioxidant and antimicrobial activities of essential oil from *Wedelia prostrata*. Excli J 12: 479-490.

Davis P.H. (1982) Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Edinburg.

de Oliveira T.M., de Carvalho R.B., da Costa I.H., de Oliveira G.A., de Souza A.A., de Lima S.G., de Freitas R.M. (2015). Evaluation of *p*-cymene, a natural antioxidant. Pharm Biol 53(3): 423-428.

Dejam M., Farahmand Y. (2017). Essential oil content and composition of Myrtle (*Myrtus communis* L.) leaves from South of Iran. J Essent Oil Bear Pl 20(3):869-872.

Duarte A., LuisA., Oleastro M., Domingues F.C. (2016). Antioxidant properties of coriander essential oil and linalool and their potential to control *Campylobacter* spp. Food Control 61: 115-122.

Elfellah M.S., Akhter M.H., Khan M.T. (1984). Anti-hyperglycaemic effect of an extract of *Myrtus communis* in streptozotocin-induced diabetes in mice. J Ethnopharmacol 11(3): 275-281.

Feisst C., Franke L., Appendino G., Werz O. (2005). Identification of molecular targets of the oligomeric nonprenylated acylphloroglucinols from *Myrtus communis* and their implication as anti-inflammatory compounds. J Pharmacol Exp Ther 315(1): 389-396

Foudil-Cherif Y., Boutarene N., Yassaa N. (2013). Chemical composition of essential oils of Algerian *Myrtus communis* and chiral analysis of their leave volatiles. J Essent Oil Res 25(5): 402-408.

Gavahian M., Farahnaky A., Javidnia K., Majzoobi M. (2013). A novel technology for extraction of essential oil from *Myrtus communis:* ohmic-assisted hydrodistillation. J Essent Oil Res 25(4): 257-266.

Habtemariam S. (2017). Antidiabetic potential of monoterpenes: A case of small molecules punching above their weight. Int J Mol Sci 19(1): 1-23.

Hayder N., Abdelwahed A., Kilani S., Ammar R.B., Mahmoud A., Ghedira K., Chekir-Ghedira L. (2004). Anti-genotoxic and free-radical scavenging activities of extracts from (Tunisian) *Myrtus communis*. Mutat Res 564(1): 89-95.

Hennia A., Miguel M.G., Brada M., Nemmiche S., Figueiredo A.C. (2016). Composition, chemical variability and effect of distillation time on leaf and fruits essential oils of *Myrtus communis* from north western Algeria. J Essent Oil Res 28(2): 146-156.

Juergens U.R. (2014). Anti-inflammatory properties of the monoterpene 1.8-cineole: current evidence for co-medication in inflammatory airway diseases. Drug Res (Stuttg) 64(12): 638-646.

Karimlar S., Naderi A., Mohammadi F., Moslehishad M., Delrish E., Aghajanpour L., Khoshzaban A., Lashay A. (2019). Hypoglycemic and hypolipidemic effects of *Myrtus communis*, *Trachyspermum*

*copticum* and *Ferula gummosa* essential oils on streptozotocin induced diabetic rats. Nutr Food Sci Res 6(1): 1-8.

Khan M., Al-Mansour M.A., Mousa A.A., Alkhathlan H.Z. (2014). Compositional characteristics of the essential oil of *Myrtus communis* grown in the central part of Saudi Arabia. J Essent Oil Res 26(1): 13-18.

Kunduhoglu B., Kurkcuoglu M., Duru M.E., Baser K.H.C. (2011). Antimicrobial and anticholinesterase activities of the essential oils isolated from *Salvia dicroantha* Stapf., *Salvia verticillata* L. subsp. *amasiaca* (Freyn and Bornm.) Bornm. and *Salvia wiedemannii* Boiss. J Med Plants Res 5(29): 6484-6490.

Lotfi P., Yaghmaei P., Ebrahim-Habibi A. (2015). Cymene and Metformin treatment effect on biochemical parameters of male NMRI mice fed with high fat diet. J Diabetes Metab Disord 14(52):2-5.

Maggio A., Loizzo M.R., Riccobono L., Bruno M., Tenuta M.C., Leporini M., Falco T., Leto C., Tuttolomondo T., Cammalleri I., La Bella S., Tundis R. (2019). Comparative chemical composition and bioactivity of leaves essential oils from nine Sicilian accessions of *Myrtus communis* L. J Essent Oil Res 31(6): 546-555.

Maxia A., Frau M.A., Falconieri D., Karchuli M.S., Kasture S. (2011). Essential oil of *Myrtus communis* inhibits inflammation in rats by reducing serum IL-6 and TNF-alpha. Nat Prod Commun 6(10): 1545-1548.

Mimica-Dukic N., Bugarin D., Grbovic S., Mitic-Culafic D., Vukovic-Gacic B., Orcic D., Jovin E., Couladis M. (2010). Essential oil of *Myrtus communis* L. as a potential antioxidant and antimutagenic agents. Molecules 15(4): 2759-2770.

Moghadam A.R.L. (2015). Antioxidant activity and chemical composition of *Rosmarinus officinalis* L. essential oil from Iran. J Essent Oil Bear Pl 18(6): 1490-1494.

More T.A., Kulkarni B.R., Nalawade M.L., Arvindekar A.U. (2014). Antidiabetic activity of linalool and limonene in streptozotocin-induced diabetic rat: A combinatorial therapy approach. Int J Pharm Pharm Sci 6(8): 159-163.

Murali R., Saravanan R. (2012). Antidiabetic effect of D-limonene, a monoterpene in streptozotocin-induced diabetic rats. Biomed Prev Nutr 2(4): 269-275.

Nassar M.I., Aboutabl E.S.A., Ahmed R.F., El-Khrisy E.D.A., Ibrahim K.M., Sleem A.A. (2010). Secondary metabolites and bioactivities of *Myrtus communis*. Pharmacognosy Res 2(6): 325-329.

Ozcan O., Ipekci H., Alev B., Ustundag U.V., Ak E., Sen A., Alturfan E.E., Sener G., Yarat A., Cetinel S., Akbay T.T. (2019). Protective effect of Myrtle (*Myrtus communis*) on burn induced skin injury. Burns 45(8): 1856-1863.

Özbek H., Yılmaz B.S. (2017). Anti-inflammatory and hypoglycemic activities of alpha-pinene. Acta Pharm Sci 55(4): 7-14.

Peana A.T., D'Aquila P.S., Panin F., Serra G., Pippia P., Moretti M.D. (2002). Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine 9(8): 721-726.

Pezhmanmehr M., Dastan D., Ebrahimi S.N., Hadian J. (2010). Essential oil constituents of leaves and fruits of *Myrtus communis* L. from Iran. J Essent Oil Bear Pl 13(1): 123-129.

Phosrithong N., Nuchtavorn N. (2016). Antioxidant and anti-inflammatory activites of *Clerodendrum* leaf extracts collected in Thailand. Eur. J Integr Med 8(3): 281-285.

Powo (2019) http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:30000914-2 (Date of access: 8 October 2019)

Rahimi M.R., Zamani R., Sadeghi H., Tayebi A.R. (2015). An experimental study of different drying methods on the quality and quantity essential oil of *Myrtus communis* L. leaves. J Essent Oil Bear Pl 18(6): 1395-1405.

Ramakrishna R., Sarkar D., Schwarz P., Shetty K. (2017). Phenolic linked anti-hyperglycemic bioactives of barley (*Hordeum vulgare* L.) cultivars as nutraceuticals targeting type 2 diabetes. Ind Crops Prod 107: 509-517.

Rossi A., Di Paola R., Mazzon E., Genovese T., Caminiti R., Bramanti P., Pergola C., Koeberle A., Werz O., Sautebin L., Cuzzocrea S. (2009). Myrtucommulone from *Myrtus communis* exhibits potent anti-inflammatory effectiveness *in vivo*. J Pharmacol Exp Ther 329(1): 76-86.

Sen A., Ozkan S., Recebova K., Cevik O., Ercan F., Kervancıoglu Demirci E., Bitis L., Sener G. (2016). Effects of *Myrtus communis* extract treatment in bile duct ligated rats. J Surg Res 205(2): 359-367.

Sen A., Yuksel M., Bulut G., Bitis L., Ercan F., Ozyılmaz-Yay N., Akbulut O., Cobanoğlu H., Ozkan S., Sener G. (2017). Therapeutic potential of *Myrtus communis* subsp. *communis* extract against acetic acid-induced colonic inflammation in rats. J Food Biochem 41(1): e12297.

Sepici A., Gürbüz I., Cevik C., Yesilada E. (2004). Hypoglycaemic effects of myrtle oil in normal and alloxan-diabetic rabbits. J Ethnopharmacol 93(2-3): 311-318.

Shahbazian D., Karami A., Eshghi S., Maggi F. (2018). Variation in the essential oil yields and compositions of Myrtle (*Myrtus communis* L.) populations collected from natural habitats of Southern Iran. J Essent Oil Res 30(5): 369-378.

Tretiakova I., Blaesius D., Maxia L., Wesselborg S., Schulze-Osthoff K., Cinatl Jr.J., Michaelis M., Werz O. (2008). Myrtucommulone from *Myrtus communis* induces apoptosis in cancer cells via the mitochondrial pathway involving caspase-9. Apoptosis 13(1): 119-131.

Tuzlacı E. (2016). Türkiye'nin Geleneksel İlaç Bitkileri. İstanbul Tıp Kitabevi, İstanbul (In Turkish)

Usai M., Mulas M., Marchetti M. (2015). Chemical composition of essential oils of leaves and flowers from five cultivars of myrtle (*Myrtus communis* L.). J Essent Oil Res 27(6): 465-476.

Wannes W.A., Mhamdi B., Sriti J., Jemia M.B., Ouchikh O., Hamdaoui G., Kchouk M.E., Marzouk B. (2010a). Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. Food Chem Toxicol 48(5): 1362–1370.

Wannes W.A., Mhamdi B., Sriti J., Marzouk B. (2010b). Changes in essential oil composition of Tunisian *Myrtus communis* var. *italica* L. during its vegetative cycle. J Essent Oil Res 22(1): 13-18.

Yu L., Yan J., Sun Z. (2017). D-limonene exhibits anti-inflammatory and antioxidant properties in an ulcerative colitis rat model via regulation of iNOS, COX-2, PGE2 and ERK signaling pathways. Mol Med Rep 15(4): 2339-2346.

Zou Y., Chang S.K., Gu Y., Qian S.Y. (2011). Antioxidant activity and phenolic compositions of lentil (*Lens culinaris* var. *morton*) extract and its fractions. J Agr Food Chem 59(6): 2268-2276.

aCS85_8