ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF LEMON BALM (*Melissa officinalis* L.) ESSENTIAL OIL

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Lemon balm (*Melissa officinalis* L.) belongs to the Lamiaceae family. Essential oil extracted from the aerial parts of lemon balm has been investigated for the protection of fruits during storage, as insecticidal, as well as in medicine, due to its bioactive properties. In this paper, the composition and identification of components from *Melissa officinalis* L. essential oil were determined by gas chromatography coupled with mass spectrophotometry (GC/MS) analysis. Total phenol content (TPC) and the scavenging activity towards 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) and 2,2'-azino-bis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS^{•+}) free radicals were evaluated by UV-VIS spectrometry. Antibacterial activities were carried out against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. Seventeen bioactive compounds were found as constituents of *Melissa officinalis* L. essential oil, among which *o*-cymene (19.735%), dehydro-*p*-cymene (17.180%), and limonene (11.589%) were found as the major components. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FT-IR) provided a confirmation for the chemical components of lemon balm essential oil identified by GC/MS. The values recorded for TPC and antioxidant activity were as follows: 54.72 mg GAE/g dry substance, 28.53% for DPPH, and 46.17% for ABTS assays, respectively. 100 μ L lemon balm essential oil proved total antibacterial activity against the tested microorganisms. The results showed that the *Melissa officinalis* L. may be a good candidate as plant-derived antioxidant and antibacterial agent for medical footwear, wound dressings and other medical applications.

Keywords: *Melissa officinalis* L., bioactive compounds, antioxidant activity, antibacterial activity

INTRODUCTION

Lemon balm (*Melissa officinalis* L.) belongs to the Lamiaceae family. *Melissa officinalis* essential oil is characterized by a fresh, lemon aroma, slightly sweet, color pale yellow and a watery viscosity. Lemon balm oil soothes and has excellent qualities in combating depression. It has a sedative effect, helps in cases of panic, slow down the heartbeat, lowers blood pressure and is a good tonic for the heart. The monoterpene oxygenated and sesquiterpenes were found as the major components of *M. officinalis* essential oil (Ghasemi Pirbalouti *et al.*, 2019). Antimicrobial and antioxidant activities of *M. officinalis* essential oil can be attributed to its polyphenol compounds. These natural antioxidants are responsible for reactive oxygen species (ROS) scavenging capacity (Lin *et al.*, 2012). In a study performed by Anastasaki *et al.* (2017) it was shown that the total phenol content (TPC) depends on the extraction solvent used in the maceration technique. Thus, in the case of plant extract with petroleum ether, the TPC was found 0.30 ± 0.03 mg of Gallic acid equivalent (GAE) per g of dry plant weight (dw), and 64.29 ± 5.70 mg/g GAE/g by using of methanol as extraction medium. Essential oil extracted from aerial parts of lemon balm has been investigated for the protection of fruits during storage (El Ouadi *et al.*, 2017), as insecticidal (Ghasemi Pirbalouti *et al.*, 2019), as well as in medicine (Lin *et al.*, 2012), due to its bioactive

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properties. However, *M. officinalis* essential oil administered in doses higher than 1 g kg^{-1} produced acute oral toxicity in rat model (Stojanović *et al.*, 2019). *M. officinalis* essential oil (0, 0.25 and 0.5% (w/v)) together with chitosan and zinc oxide have been used for the preparation of antimicrobial biodegradable composite film having food packaging application, due to the good antimicrobial activity against *E. coli*, antioxidant activity and mechanical properties (Sani *et al.*, 2019).

MATERIALS AND METHODS

Material

Lemon balm (*Melissa officinalis* L.) essential oil was acquired from Solaris Plant SRL-Radix (Darmstadt, Germany). Cotton material was used for impregnation with different concentrations of essential oils in order to simulate the footwear lining or bandages. Other chemical reagents for analysis were analytical grade.

Methods

Preparation of Tested Materials

Discs of cotton samples (20 mm diameter cotton discs, three for each sample) were treated with different amounts of lemon balm essential oil: 30 μL , 50 μL , and 100 μL , labeled as BBCR₃₀, BBCR₅₀, and BBCR₁₀₀, respectively. Cotton samples without essential oil were used as control.

GC/MS Analysis

In order to determine the bioactive compounds from lemon balm essential oil, a Thermo Scientific gas chromatograph coupled with mass spectrometer, DSQ II MS, equipped with TR-5 MS non-polar capillary column ($60 \text{ m} \times 0.25 \mu\text{m} \times 0.25 \mu\text{m}$) was used. The temperature was set in the range between room temperature to 350°C , heating rate was programmable between 0.1 and $120^\circ\text{C}/\text{min}$, temperature of split/splitless injector was in the range of 50°C and 375°C , and pneumatic control system of pressure/carrying helium gas flow was of 5%.

ATR-FTIR Spectroscopy

ATR-FTIR analysis was performed with a FT-IR/ATR spectrometer-Jasco 4200 operating in the range of 4000 to 600 cm^{-1} , with a spectral resolution of 0.5 cm^{-1} .

Determination of Total Phenol Content (TPC)

The total phenol content was determined using Folin-Ciocalteu method. To 0.5 mL of the sample, 3.6 mL H_2O , and 0.24 mL Folin-Ciocalteu reagent were added. After 5 min, 0.68 mL of 7.5% Na_2CO_3 were added and the resulted solution was mixed and allowed to stand for 30 min at 40°C . The absorbance at 750 nm was measured using a UV-Vis spectrophotometer, and calculated based on a calibration curve with Gallic acid (0-500 mg/mL, $R^2 = 0.995$) previously established. The total phenol content was expressed as Gallic acid equivalent (GAE) in mg/g of dry weight.

DPPH Radical-Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity was evaluated using the method proposed by Sharma *et al.* (2017). 2.5 mL of DPPH solution (100 µM obtained by dissolving in ethanol) was added to 500 µL of sample (concentration of 60 mg/mL). The mixture resulted was shaken vigorously and kept in the dark for 30 min at room temperature. The decrease in absorbance of the analyzed solution at 517 nm was monitored using an UV-Vis spectrophotometer. Radical scavenging antioxidant activity of sample was calculated according to the equation:

$$\text{DPPH free radical scavenging activity (\%)} = [(A_c - A_s)/A_c] \times 100 \quad (1)$$

where: A_c – absorbance of control, A_s – absorbance of sample.

ABTS Free Radical Scavenging Assay

The experiment was carried out using an improved ABTS discoloring assay (Huang *et al.*, 2017). The 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) radical cation (ABTS^{•+}) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark for at least 16 h at room temperature prior to use. The ABTS^{•+} solution was diluted to an absorbance of 0.7 ± 0.05 at 750 nm. 140 µL of essential oil (60 mg/mL) were mixed with 4 mL ABTS^{•+} solution and kept in the dark, at room temperature for 6 min. The affinity of essential oil to quench ABTS free radical was evaluated according to the following equation:

$$\text{ABTS free radical scavenging activity (\%)} = [(A_c - A_s) / A_c] \times 100 \quad (2)$$

where: A_c – absorbance of control, A_s – absorbance of sample.

All the experiments were carried out in triplicates ($n = 3$) for each sample and mean average values with standard deviation ($\pm$ SD) were reported.

Microbiological Analyses

Microbiological analyses were performed according to ISO 20743:2013, *Textiles — Determination of antibacterial activity of textile products*, the absorption method.

RESULTS AND DISCUSSIONS

The composition and bioactive compounds from lemon balm essential oil were determined by GC-MS analysis. The major compounds found in lemon balm essential oil were (% of area): 19.735% of *o*-cymene, 17.180% of dehydro-*p*-cymene and 11.589% of limonene. In this study, seventeen bioactive compounds were detected as constituents of *Melissa officinalis* essential oil representing more than 50% of the composition. El Ouadi *et al.* (2017) reported the P-mentha-1,2,3-triol (13.1%), P-menth-3-en-8-ol (8.8%), piperitenone oxide (8.4%) and Z-piperitone oxide (7.3%) as the main compounds identified in *M. officinalis* essential oil. A comparison of our results with the previous reports (Ghasemi Pirbalouti *et al.*, 2019; Stojanović *et al.*, 2019) evidenced some differences in the volatile composition of the plant assigned to the geographic origin of the plant sample, methods of extraction, and environmental factors.

The ATR-FTIR spectrum showed in Figure 1 reveals the spectral bands at: 2920 cm⁻¹, 2857 cm⁻¹ (aliphatic C-H), 1727 cm⁻¹, 1673 cm⁻¹, 1632 cm⁻¹, 1444 cm⁻¹ (C=C stretching vibration of the aromatic ring from *p*-cymene), 1379 cm⁻¹ (isopropyl methyl group symmetric bending vibration), 1232 cm⁻¹ (C–O–C stretching), 1191 cm⁻¹, 1154 cm⁻¹,

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1118 cm^{-1} (CH_3 deformations), 1022 cm^{-1} , 985 cm^{-1} (para-substituted phenyl) (Valderrama and Rojas De, 2017), 885 cm^{-1} , 841 cm^{-1} (C-H bend pattern from dehydro-*p*-cymene and limonene), 743 cm^{-1} , (aromatic C-H out-of-plane bend from *o*-cymene).

Figure 1. ATR-FTIR spectrum of *Melissa officinalis* essential oil

The *M. officinalis* essential oil was evaluated for the total phenol content and antioxidant activities by using DPPH and ABTS radical scavenging bioassays. The results are shown in Table 1.

Table 1. TPC and the radical scavenging activity results for *M. officinalis* essential oil

TPC (mg GAE/g dry substance)	DPPH free radical scavenging activity (%)	ABTS free radical scavenging activity (%)
54.72±4.8	28.53±5.4	46.17±4.7

The results from Table 1 prove the potential of *M. officinalis* essential oil to be used as antioxidant agent. The total phenol content is comparable with similar reported values for extracts in petroleum ether (Lin *et al.*, 2012; Anastasaki *et al.*, 2017). Antibacterial properties were performed against *Staphylococcus aureus* ATCC 6538 (Table 2), *Escherichia coli* ATCC 10536 (Table 3) and *Klebsiella pneumonia* ATCC 4352 (Table 4).

Table 2. Resistance against *Staphylococcus aureus* of cotton samples treated with *M. officinalis* essential oil

Sample	Images	T ₀ (UFC/mL)	T ₂₄ (UFC/mL)	R (%)	Log ₁₀
Inoculum concentration		1x10 ⁵	-	-	-
Control		1x10 ⁵	1.115x10 ³	98.88	1.95
BBCR ₃₀		1x10 ⁵	3.5x10 ²	99.65	3.46
BBCR ₅₀		1x10 ⁵	1.6x10 ¹	99.98	3.79
BBCR ₁₀₀		1x10 ⁵	4	100	4.4

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Table 3. Resistance against *Escherichia coli* of cotton samples treated with *M. officinalis* essential oil

Sample	Images	T ₀ (UFC/mL)	T ₂₄ (UFC/mL)	R (%)	Log ₁₀
Inoculum concentration		1x10 ⁵	-	-	-
Control		1x10 ⁵	1.12x10 ³	98.88	1.96
BBCR ₃₀		1x10 ⁵	5.44x10 ²	99.46	2.26
BBCR ₅₀		1x10 ⁵	1.6x10 ¹	99.98	3.79
BBCR ₁₀₀		1x10 ⁵	1	100	5

Table 4. Resistance against *Klebsiella pneumonia* of cotton samples treated with *M. officinalis* essential oil

Sample	Images	T ₀ (UFC/mL)	T ₂₄ (UFC/mL)	R (%)	Log ₁₀
Inoculum concentration		1x10 ⁵	-	-	-
Control		1x10 ⁵	7.2x10 ²	99.2	2.14
BBCR ₃₀		1x10 ⁵	7x10 ¹	99.93	3.15
BBCR ₅₀		1x10 ⁵	2.4x10 ¹	99.98	3.62
BBCR ₁₀₀		1x10 ⁵	0	100	5

The results from Tables 2-4 showed that the cotton samples treated with 100 µL lemon balm essential oil have 100% resistance against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*.

CONCLUSION

Melissa officinalis L. essential oil was investigated for its antioxidant by TPC, DPPH and ABTS methods and showed 54.72 mg GAE/g dry substance, 28.53% for DPPH, and 46.17% for ABTS assays, respectively. The antibacterial activity tested against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* on cotton samples treated with different concentrations of essential oil proved 100% UFC reduction for samples treated with 100 µL of essential oil. The main bioactive compounds detected by GC-MS, *o*-cymene, dehydro-*p*-cymene and limonene are responsible for the high antioxidant and antibacterial activities. *Melissa officinalis* L. essential oil could be used as potential bioactive agent for medical footwear, wound dressings and other medical applications.

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