Industrial Crops & Products 112 (2018) 783–789

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Cultivar affects the phenolic composition and antioxidant properties of commercially available lemon balm (*Melissa officinalis* L.) varieties

Maxime M. Boneza, Emily D. Niemeyer*

Department of Chemistry and Biochemistry, Southwestern University, 1001 E. University Ave., Georgetown, TX, 78626, USA

ARTICLE INFO

Keywords:
Phenolic compounds
Antioxidants
Lemon balm
Lamiaceae family
Melissa officinalis

ABSTRACT

Although agronomic factors affecting essential oil composition within lemon balm (*Melissa officinalis* L.) are well documented, much less is known about conditions that influence foliar phenolic levels. Therefore in this study, the effect of cultivar and seed company on the phenolic composition and antioxidant properties of five commercially available lemon balm varieties was determined. Cultivar ($p = 0.009$) and seed company ($p = 0.020$) had a significant effect on total phenolic concentrations. Analysis of individual phenolic acid levels by high-performance liquid chromatography showed substantial variations in the phenolic acid composition among cultivars. Rosmarinic ($p = 0.001$), gentisic ($p = 0.014$), chicoric ($p < 0.001$), protocatechuic ($p = 0.018$), and *p*-coumaric ($p < 0.001$) acid concentrations were affected by cultivar, although caftaric, caffeic and gallic acids were not. Seed company was a significant factor influencing rosmarinic ($p = 0.007$), gentisic ($p = 0.002$), chicoric ($p = 0.034$) and *p*-coumaric ($p = 0.024$) acid content. Cultivar also had a significant influence on FRAP (ferric reducing antioxidant power, $p = 0.028$) and DPPH (2,2-diphenyl-1-picrylhydrazyl, $p = 0.002$) antioxidant capacities. These results suggest that both the selection of cultivar and seed origin are important factors influencing the concentration of phenolic compounds and the resulting antioxidant properties within lemon balm.

1. Introduction

Lemon balm (*Melissa officinalis* L.) is a medicinal herb of the Lamiaceae family that is native to the Mediterranean and is now cultivated throughout Europe, North America, and Asia. In traditional medicine, lemon balm is commonly administered as a tea infusion that is used to treat maladies such as gastrointestinal complaints, headache, and fever (DerMarderosian and Beutler, 2014; Shakeri et al., 2016). Lemon balm essential oil exhibits antibacterial and antifungal properties while aqueous extracts have strong antiviral activity (Bruneton, 1999). Like other Lamiaceae herbs, lemon balm contains high concentrations of phenolic acids, particularly caffeic acid derivatives such as rosmarinic acid (Barros et al., 2013; Fecka and Turek, 2007; Milevskaya et al., 2017). These phenolic acids likely contribute to lemon balm's medicinal properties and have been associated with the herb's high antioxidant capacity (Dastmalchi et al., 2008; Proestos et al., 2005; Skotti et al., 2014) as well as anti-proliferative (Lin et al., 2012) and antiprotozoal (Cunha et al., 2016) activities.

Lemon balm has been used as a medicinal remedy since ancient times (DerMarderosian and Beutler, 2014; Shakeri et al., 2016), yet few studies have examined agronomic factors that may increase phenolic content – and maximize antioxidant properties – of this important herb. Yadegari (2017) reported that foliar application of key micronutrients (Zn, Fe, Mn, and Cu) during plant development increased both phenolic levels and essential oil content in lemon balm. By comparing lemon balm grown under light emitting diodes (LEDs) and fluorescent bulbs, Frąszczak et al. (2015) found that spectral composition influenced lemon balm's phenolic production, with lemon balm grown under LEDs having significantly higher essential oil content and phenolic acid content. Engel et al. (2016) determined that both biomass and rosmarinic acid content in lemon balm increased significantly when plants were inoculated with arbuscular mycorrhizal fungi. Most recently, Pereira and co-workers (2018) reported that irradiating dried lemon balm post-harvest with gamma or electron-beam radiation led to increased levels of particular phenolic acids.

Although factors affecting essential oil composition in lemon balm are well documented (Frąszczak et al., 2015; Szabó et al., 2016; Yadegari, 2017), much less is known about conditions that influence foliar phenolic levels in lemon balm. Genetic factors such as cultivar can play an important role in the production of phenolic compounds within some plants (Scalzoa et al., 2005; Parr and Bolwell, 2000). For example, Szabó et al. (2016) studied five lemon balm genotypes and

* Corresponding author.
E-mail addresses: maximejn@hotmail.com (M.M. Boneza), niemeyee@southwestern.edu (E.D. Niemeyer).

https://doi.org/10.1016/j.indcrop.2018.01.003
Received 5 October 2017; Received in revised form 1 December 2017; Accepted 3 January 2018

found significant variability in chemical composition among varieties, with the 'Lorelei' cultivar having both the highest essential oil and rosmarinic acid content while 'Soroksár' lemon balm had the highest measured antioxidant capacity. The purpose of the current study is to determine how the phenolic acid content and corresponding antioxidant properties vary among five common lemon balm cultivars. Specifically, similarities and differences in total leaf phenolic concentrations, individual phenolic acid profiles, and measured antioxidant capacities were examined among commercially available lemon balm varieties: 'Citronella,' 'Quedlinburger Niederliegende,' 'Lime,' 'Lemonella,' and common lemon balm cultivars. Additionally, the influence of seed source on phenolic content and antioxidant properties was determined. Because phenolic levels correlate strongly with antioxidant properties (Rice-Evans et al., 1997), understanding variations in phenolic content among commercially purchased lemon balm cultivars will ultimately allow growers to select and cultivate varieties with maximum phenolic content and corresponding health benefits.

2. Materials and methods

2.1. Chemicals and reagents

Analytical standards including rosmarinic acid (97%), chicoric acid (> 95%), caffeic acid (> 98%), protocatechuic acid (> 97%), gallic acid (> 98%), gentisic acid (> 99%), *p*-coumaric acid (> 98%), caftaric acid (> 97%), and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, > 98.0%) were of the highest purity and were purchased from Sigma Aldrich (St. Louis, MO, USA). Other reagents such as sodium carbonate, TPTZ (2,4,6-tripyridyl-s-triazine), DPPH (2,2-diphenyl-1-picrylhydrazyl), hydrochloric acid, iron (III) chloride hexahydrate, sodium acetate, and formic acid were also purchased from Sigma Aldrich. Folin-Ciocalteu reagent was obtained from VWR International (West Chester, PA, USA). Acetonitrile and methanol were high-performance liquid chromatography (HPLC) grade and were purchased from Pharmco-AAPER (Brookfield, CT, USA).

2.2. Experimental design

Five cultivars of lemon balm seeds were purchased from three different companies: 'Citronella' and 'Quedlinburger Niederliegende' from Richters Herbs (Goodwood, ON, Canada) and Swallowtail Garden Seeds (Santa Rosa, CA, USA); 'Lime' from Terroir Seeds (Chino Valley, AZ, USA); 'Lemonella' from Richters Herbs, and common lemon balm from Terroir Seeds and Richters Herbs. Seeds were sown directly in seed trays containing Ferti-Lome Ultimate Potting Mix (Cheek Garden Products, Austin, TX, USA) within the Southwestern University greenhouse. Eighteen days after sowing, seedlings were transplanted into 1-gallon plastic pots containing Ferti-Lome Ultimate Potting Mix then placed in a randomized block design consisting of five replicate plants per cultivar from each seed company (n = 40 plants total). Plants were grown in the greenhouse under natural light and temperature conditions (temperature range: 23.6–39.6 °C) for 38 days. Watering occurred twice a day and the plants were fertilized once per week with a Miracle-Gro All Purpose Plant Food watering solution (The Scotts Miracle-Gro Company, Marysville, OH, USA).

2.3. Sample preparation

Leaves were harvested from individual plants, immediately frozen in liquid nitrogen, and stored at −80 °C until processing. The leaves were crushed in liquid nitrogen using a mortar and pestle then dried for 3.5 h by vacuum centrifugation. Dried samples were first weighed to obtain their mass then incubated in 1.0 mL of 80% aqueous methanol with shaking for a period of 15 h. Solutions were centrifuged at 12,000 rpm for 20 min at room temperature and the supernatant was filtered then stored at −80 °C for further analysis.

2.4. Determination of total phenolic content

Total phenolic content for each sample was measured using a modified version of the Folin-Ciocalteu assay (Nguyen and Niemeyer, 2008). A mixture of 150 µL of water, 100 µL of 80% methanolic lemon balm extract, 125 µL of Folin-Ciocalteu reagent, and 625 µL of 0.2 M aqueous sodium carbonate were incubated at room temperature for 20 min. Mixtures were centrifuged at 13,200 rpm for 10 min and the absorbance of the supernatant was measured at 735 nm in triplicate on a microplate reader (Biorad Benchmark, Hercules, CA, USA) against a blank consisting of 150 µL of water and 100 µL of 80% aqueous methanol. The sample absorbance was compared with a gallic acid standard curve (20–800 mg/L) and concentrations were reported in gallic acid equivalents (GAE) in mg/g dry weight (DW).

2.5. Determination of antioxidant properties

The antioxidant reducing capacity of each lemon balm extract was determined using a modified version of the FRAP assay (Benzie and Strain, 1996). FRAP solution was prepared daily and contained 20 mL of acetate buffer (0.3 M, pH 3.6), 2 mL of 20 mM aqueous iron (III) chloride hexahydrate, and 2 mL of 10 mM TPTZ dissolved in 40 mM hydrochloric acid. For sample preparation, 25 µL of lemon balm extract was incubated with 250 µL of FRAP reagent in a 96-well microplate, shaken at room temperature for 8 min, and the absorbance was measured at 593 nm. The control consisted of 25 µL of 80% aqueous methanol and 250 µL of the FRAP reagent. The blank-subtracted sample absorbance was compared with a trolox standard curve (107–1000 µM) and the concentrations were reported in trolox equivalent antioxidant capacity (TEAC) in µmol/g DW.

The antioxidant capacity was also studied using a modified version of the DPPH free radical scavenging assay as described by Cheng et al. (2006). All samples were diluted prior to analysis. Methanolic lemon balm extract (400 µL) and 0.2 µM DPPH in 50% acetone (400 µL) were mixed thoroughly and incubated in the dark for 45 min at room temperature. The absorbance of the sample mixture (A_{sample}) and the absorbance of a control ($A_{control}$, which contained 400 µL of 80% methanol and 400 µL of 0.2 µM DPPH in 50% acetone) were monitored at 515 nm. The absorbance of a blank sample (A_{blank}) containing 80% methanol was also analyzed. The % DPPH free radical scavenging was then calculated according to the following equation: $\%\ \text{DPPH free radical scavenging} = (1 - [(A_{sample} - A_{blank})/(A_{control} - A_{blank})]) \times 100$. The antioxidant capacity was determined by comparing the % DPPH free radical scavenging of each lemon balm sample to a trolox calibration curve (10–150 µmol) and concentrations were reported in µmol TEAC/g DW.

2.6. Quantification of individual phenolic acids

A dual-pump Waters 717 Plus Autosampler HPLC system (Milford, MA, USA) equipped with a Phenomenex (Torrence, CA, USA) Kinetex C-18 column (2.6 µm, 4.6 mm × 100 mm) was used for the identification and quantification of individual phenolic acids. Detection wavelengths were 280 and 330 nm and the sample injection size was 10 µL. Eluent A was 3% methanol and 1% formic acid in water and eluent B was 0.1% formic acid in acetonitrile. The following linear gradient was used with a mobile phase flow rate of 0.6 mL/min: 95% A, 0 min; 95% A, 2 min; 75% A, 12 min; 75% A, 17 min; 10% A, 18 min; 10% A, 23 min, 95% A, 24 min. Eight phenolic acid standard solutions (caffeic acid, caftaric acid, chicoric acid, *p*-coumaric acid, gallic acid, gentisic acid, protocatechuic acid, and rosmarinic acid) were prepared over a concentration range of 2.9–75.0 mg/L and individual calibration curves were constructed (with $r^2 \geq 0.99$ to ensure linearity). Method accuracy and reproducibility were determined by repeated injections of a mixed phenolic acid standard of known concentration during a given HPLC analysis. Phenolic acids within individual lemon balm extracts were